

Can British universities recover?

The British government seems to be repenting its six years of meanness towards higher education, but the decay it engendered may by now be irreparable.

CURIOUS things are happening in British higher education. Suddenly, again, access is the cry. That, at least, was one of the themes with which Mr Kenneth Baker, Secretary of State for Education and Science for the past four months, tempted university vice-chancellors at their annual retreat last week (see page 383). Does not Britain need more skilled people, of the kind that universities might train. Is not the prospective decline of the late teenage groups an opportunity to cast the net of education wider? And what is this depressed talk of having to close one or more universities for lack of funds? Mr Baker, always emollient, seems to have been almost chirpy. Many in his audience, hearing their own arguments advanced by their paymaster, must have wondered whether they had been dreaming all those years. The beginning of the new term (these things start late in Britain) will remind them that their nightmare has been reality as well.

Mr Baker may unintentionally have prolonged the agony by acknowledging that British universities need more money to stay afloat and then going on to deal chiefly with the mechanism by which students might be supported. True, he half-promised that there will be more money in next year's budget, for which many people will be grateful. But, for at least the third time since it was first elected in 1979 the British Government is once again dicker with schemes for supporting students by means of loans. Mr George Walden, an able colleague of Mr Baker's, is well launched into an enquiry on the subject. But to judge from the hints Mr Baker dropped last week the government is thinking dangerously big.

It is one thing to devise a scheme by which the costs of maintaining students in higher education can be met by some mixture of grants and loans, and quite another thing to talk (as Mr Baker did) of shuffling off a substantial part of the cost of running universities onto the employers of university graduates. The former would have the advantage of freeing the government from the need to write a cheque each year determined by the numbers of students in higher education; the second would have the disadvantage of giving employers an unreasonable influence over the lives and working conditions of university-leavers. For at least as long as Britain remains as impoverished as it is, the government has no equitable choice but to meet the core costs of higher education out of taxpayers' resources. That could change, but only if universities first prosper.

What are the chances of that? Not bright. Although Mr Baker went last week as far as could reasonably be expected of a government minister to disavow the policies of his predecessor, Sir Keith Joseph, even to the extent of acknowledging that last year's policy document on the future of higher education was disastrous, he may not yet be fully aware of how six years of attrition have undermined the system he now wishes to encourage. Too many able academics have been lost to the system, while the long period in which new recruitment has been impossible has robbed universities of their necessary complement of intellectual subversives. The consequences are apparent to all who spend time counting the places within the British university system at which innovative people prosper; fewer fingers are needed as the years go by. It could be that there are already too

few places at which outstanding research is practised for the British system as a whole to have the vitality to sustain Mr Baker's rediscovered goals.

The best hope is that the universities themselves will come to recognize that they have an interest in, and a responsibility for, their own survival. On the narrow question of student maintenance, it has taken them ten years (until now) to acknowledge that the government has had a good case for shading the principle of a grant for everybody with a place in higher education. They would have won a better deal if they had said so sooner. Even now, while struggling with the government's spate of requests for better management, fuller accountability, less tenure and better student behaviour (under the legend of "free speech"), the universities wishing to survive should recognize that that prize will go to those with the wit to take the initiative. One immediate objective should be a measure of financial integrity. Too many universities in Britain are over-spending their over-modest budgets in ways that put them in hock to the University Grants Committee and even, on some occasions, the commercial banks; no institution can expect to make an independent way in the world in such a condition. Further ahead, universities need a measure of the autonomy most of them at present lack. In practice, what this means is that they should be more free to teach what they deliberately decide they must teach, and that they will pursue research in the fields best suited to the talents and interests of their members. Present circumstances cramp this freedom, but British universities have been extraordinarily compliant of these restraints. Is that another sign that the stuffing has gone out of them? □

Ideal undermines good

The Soviet Union gives the United States too little credit for a shift on arms control.

FOR most of this year, the Soviet Union has made most of the running in progress towards arms control, portraying the United States as a laggard in the process, but last week's performances at the United Nations by President Reagan (on Monday) and Mr Eduard Shevardnadze, the Soviet Foreign Minister (on Tuesday), may have tilted the balance the other way. As on previous occasions, but through a different spokesman, the Soviet Union hotly rejected President Reagan's statement of the present position of the United States on arms control as so much "propaganda". That is unfortunate because Mr Reagan's speech embodies signs of change and relaxation on at least two important issues, the Strategic Defence Initiative (SDI) and the prospects for a nuclear test-ban. In neither case were the US concessions enough to bridge the well-known gap between the United States and the Soviet Union, but that does not imply that they are negligible.

On SDI, the present position is that Soviet Union has asked for a 15-year extension of the Anti-Ballistic Missile Treaty (which would prohibit the deployment of SDI weapons), but has said that laboratory research on defences against ballistic mis-



siles are allowable. The United States has said that the research will go ahead, but that it will offer to "share" whatever technology emerges before anything is deployed. Mr Reagan appears to have offered something of a compromise last week by saying that, if there is an agreement on "radical reductions" in strategic weapons, the United States would agree to keep within the limits of the ABM treaty until the end of 1991, and would then allow two years for a period of negotiation with the Soviet Union on a plan "for sharing the benefits" of SDI technology with the Soviet Union. Failure to agree would allow either side to give six months' notice of deployment.

Mr Shevardnadze's reply the following day was direct, unnecessarily so: SDI is being developed in the United States with the objective of making a first-strike against the Soviet Union without fear of retaliation, the period for negotiations outlined by Mr Reagan coincides with that required to complete work on SDI and, in general, "evil designs are being passed off as good intentions". But Mr Reagan has been almost more forthcoming now than ever in the past six years, while his forms of words suggest room for negotiation and accommodation. In any case, the Soviet response leaves out of account the likelihood that full-blown SDI is unattainable for technical reasons, that an effective early-warning against attack may nevertheless emerge and that the US Congress will continue to keep SDI on a tighter rein than the US administration would like. Especially because Mr Reagan's successor two years from now is unlikely to follow willingly in his predecessor's path, there is surely scope for a deal that would satisfy both sides.

Test-ban

On the test-ban, President Reagan outlined two important changes. First, he said, the United States is now ready to ratify the existing treaties which ban even underground tests of nuclear weapons yielding more than 150,000 tonnes of TNT equivalent and which prohibit the use of nuclear explosions for peaceful purposes except under stringent conditions unlikely ever to be satisfied. (High time, it may be said; all this could and should have been done five years ago.) Second, the United States, according to its president, is willing "to implement a step-by-step parallel programme of limiting and ultimately ending" nuclear tests. This may not be the immediate agreement to end tests for which the Soviet Union has been asking for the past several months, but it is a long step ahead of the position of the United States for the past few months that, while nuclear stockpiles remain, tests will be needed to make sure they are effective. The previous position is logically untenable but also immune to negotiation. The programme outlined last week is a starting-point for talks.

The Soviet position, as defined last week, sounded shrill by comparison. It is true that Mr Shevardnadze advocated the benefits of a bilateral moratorium on tests which would eventually be made "multilateral", chiefly to prevent the emergence of "new kinds" of nuclear weapons (bomb-driven X-ray lasers?). So far, so good. But why go on to say that the goal must be the "scrapping of all nuclear weapons" when even Mr Mikhail Gorbachev (in the proposals on the subject last January) seemed to acknowledge that such a step can only be the culmination of a long process of negotiation, a large part of which is directed at making sure that the two sides understand each other again? To judge from this section of his speech, Mr Shevardnadze may have thought that he was addressing the annual assembly of the British Liberal Party, which that very day embarrassed its leader, Mr David Steele, by voting (narrowly) in favour of (vaguely) unilateral nuclear disarmament. The plain truth is that, while further steps towards arms control have become urgently necessary, nobody anywhere, neither in Moscow nor Washington, has yet given thought to the problem of what a world free from nuclear weapons would be like and that, meanwhile, the abolition of nuclear weapons would probably make the world more dangerous, not less. That is not a serious negotiating position. □

Computers by halves

The United States should abandon attempts to blame financial instability on computers.

WHEN railway locomotives came on the scene, the people who flocked to avail themselves of the benefits also complained about the noise and the smoke and more than a century has passed without these nuisances being fully abated. Now it is the same with drugs; people want to be cured of disease, but believe that side-effects should be abolished. The financial community in the United States seems to be stumbling in the same direction. Last week, the Securities and Exchange Commission (SEC), which zealously regulates financial markets, embarked on an investigation whose objective seems to be to pin responsibility for the sudden collapse of Wall Street on 11 September this year (*Nature*, 323, 190; 1986) on the now widespread use of computers in the buying and selling of financial assets. This development is wayward, to say the least; nobody knows better than SEC itself that Wall Street would physically collapse without the computers to which stockbrokers have grown accustomed. Earlier this year, SEC brought off a great coup by using computers to find evidence for insider trading on Wall Street. So what cause can there be for welcoming computers as a general aid to efficiency while, at the same time, seeking to cramp their use in some parts of the market?

The starting-point for SEC's luddite investigation is the belief that what is called program trading may have accelerated the fall of stock prices two weeks ago. The principle is that computers should be programmed to buy or sell particular financial securities when predetermined circumstances obtain; the machines can even be programmed to print out the forms by which orders to buy or sell are contractually confirmed.

What worries SEC is that, two weeks ago, some of the selling of stocks may have been executed by computers programmed to compare the prices of particular stocks on Wall Street with the prices of options to buy or sell the same paper commodities a few months later, the market for which is based chiefly in Chicago. Opportunities for trouble arose because prices were falling so rapidly in New York that the options market in Chicago could not adjust its prices quickly enough. So clever New York computers were able to make a killing by selling stocks and, at the same time, buying or selling options, no doubt to the profit of their owners. By the law of the conservation of money (which is valid in the short run) there would have been losers as well, the options traders in Chicago, one of whom is reported to have lost more than \$10 million on 11 September.

Nobody suggests that the sale of options to buy or sell stocks should itself be abolished. The practice, the basis for the most rapid growth in financial markets in the past few years, is both sensible and valuable. A nervous holder of some stock may, for example, buy for a modest price an option to sell the same stock three months ahead at a predetermined price. If, in the interval, the price of the stock should fall, he will be protected from that decline for the modest price of the option. If, in the event, the price does not fall, he can liquidate the option to sell by buying, nearer the time, an offsetting contract to buy the same quantity of the same stock. Not only would it be inequitable to restrict these arcane manoeuvres, but impossible as well; other financial centres would be delighted to take over from Chicago. But, similarly, there can be no case in equity for cramping the efficiency with which licensed traders use machines to execute their orders to buy and sell. If there is a lesson to be learned from what happened on 11 September, it is that the options traders in Chicago should get themselves better machines. The moral is what it has always been, that even if the collapse of prices on 11 September was helped along by programmed trading, the cause was the persisting uncertainty about the economic future. Fiddling with restriction on computers will not get rid of that. □

British universities

More money and more trouble ahead

Mr Kenneth Baker, Secretary of State for Education and Science in the British government, last week offered a mixture of sticks and carrots to British academics. In his first major statement of higher education since his appointment in the spring, Mr Baker seems to have left the universities with the impression that he is both on their side and on that of the government.

In a speech to the otherwise private annual meeting of the Committee of Vice-Chancellors and Principals at Edinburgh, Mr Baker said that there would be more money in the coming year (although the amount will not be known for some weeks) and that he, and the government of which he is a member, were now committed to wider access to higher education for students of all ages.

But Mr Baker also reminded universities of their obligations, in particular the need to concentrate research effort on the stronger departments, the "rationaliza-

tion" of small departments, the need for better management and teaching quality as well as the abridgement of tenure. Using the word "accountability" only in quotation marks, he asked universities to welcome public scrutiny of their affairs, not least because of "the opportunity it also brings to create public support".

The most striking feature of Mr Baker's statement is the declaration of his commitment to wider access, which comes after a long period when the government has sought to control the cost of higher education by restricting student numbers. Last week, the minister said that he wants to see "a higher proportion of our young people, and more older students, going into higher education of all kinds". He said that contraction "simply does not square" with Britain's need for highly qualified manpower.

The other side of the coin is the question of how the system might be paid for. With

the prospect that the cost of the British National Health Service will increase at 1 per cent a year simply to keep pace with demographic change, Mr Baker argued that higher education could not take more out of the economy in the long run than its present £3,000 million a year.

Student loans appear again to be part of the British government's answer to this long-standing problem although Mr Baker acknowledged that change from the present system of mandatory but means-tested maintenance grants for students would take time. His remarks coincided with the discussion by the vice-chancellors of a working paper in which one of the vice-chancellors' own committees had reluctantly come to the conclusion that loans might provide the quickest relief from the present impoverishment of many British students.

Mr Baker went much further, however, by arguing that the cost of higher education should be shared more equally between the government and parents on the one hand and by students and employers on the other. In particular, "we need to consider how employers might be given a more fundamental stake in the education and professional development of their future employees". □

Strategic exports

High-technology export tangle

Washington

IS THERE a pipeline for contraband high technology running from Columbus Ohio straight to Moscow? Possibly not, but that has not stopped the US Customs Service from holding for 15 months equipment worth \$50,000 designed to measure respiration in rats pending the outcome of an investigation of possible export law violations. Jan Czekajewski, president of Columbus Instrument, the company that makes the equipment, is anxious to prove that not only did he violate no laws, but that selling his oxygen analysers to Eastern European countries does not pose a threat to national security.

Czekajewski's troubles began in early 1985. His European distributor, the Finnish firm Anitek, requested samples of Columbus Instrument's latest equipment for a trade show in Moscow. On 30 May 1985, equipment worth \$228,812 was consigned to Burlington Air Freight for shipment to Finland. But on 1 June, at New York's Kennedy Airport, customs agents seized the shipment. They found the analysers, but they also found six computers; four Apple-IIe's, one Rockwell AIM-65 and one "SuperComputer", an IBM-PC clone made in Taiwan.

No export licenses were sought for the shipment as Czekajewski assumed that it did not require one. (The computers were hardly state-of-the-art, while the oxygen

analysers never required any special licence.) Two days later, customs agents went to Columbus Instrument headquarters, searching for other computer and high-technology equipment. Czekajewski says the customs agents made a second visit on 13 June, this time accompanied by reporters and television news crews. Local news broadcasts that night spoke of Customs agents unearthing a plot to ship "supercomputers" and other high-technology equipment to the Soviet Union.

That was more than a year ago. So far, no formal charges have been filed against Czekajewski. In October, the Commerce Department's International Trade Administration determined that all computers in the shipment but the SuperComputer did not require a special license and should be returned to Columbus Instruments. The Customs Service returned most of the shipment in May. Still being held is one oxygen consumption monitor, controlled by the Super Computer.

Customs agents claim that shipping documents accompanying Czekajewski's equipment were misleading. The documents stated the final destination for the shipment was Finland, not Moscow. Czekajewski explains that his company typically lists its distributor's address as a shipment's final destination, since it is never certain to whom it will be sold.

At issue still is whether the SuperCom-

puter operates faster than permitted for unlicensed exports to eastern European countries. The Commerce Department says it does, but it is evaluating the companies claims to the contrary. Czekajewski argues that as the computer is from Taiwan, it should not be subject to US export controls and says it is possible to buy an equivalent computer in Bulgaria.

Information about the case is not easy to come by. The US attorney's office in Ohio will not comment because the case is before a grand jury. Neither will the Customs Service. The Commerce Department says the National Bureau of Standards is testing the SuperComputer to see if it exceeds allowable performance standards, but a spokesperson for the National Bureau of Standards denies that it is doing any such testing.

In August Customs Service associate commissioner Richard Miller wrote to Czekajewski's congressional representative Senator Howard Metzenbaum (Democrat, Ohio) saying that "the terms national security and supercomputer are not issues" in this case, and that the Customs Service is merely attempting to determine whether export laws were deliberately broken. But for Czekajewski, who emigrated to the United States from Poland 18 years ago, the experience has been a nightmare. His message for others that he now stamps on his correspondence: "WARNING! Exporting can be hazardous to your mental health."

Joseph Palca

AIDS in California

Proposition causes PANIC

San Francisco

DO THE people of California know better than state health officials how best to control the spread of acquired immune deficiency syndrome (AIDS)? Supporters of Proposition 64, an initiative on the California ballot in the November elections, believe they do. If the proposition commands a majority in November, it will require the state to extend to AIDS the regulations now applying to other infectious diseases. Meanwhile, the proposition is opposed by health care professionals and the opponents of the extreme right.

According to the proposition's sponsors, the ballot initiative merely "extends existing public health codes for communicable diseases to AIDS and AIDS virus carriers". But opponents of the measure, including many state officials and health care professionals, believe the proposition is deceptive, and may only increase the threat of AIDS.

Among those who have arranged that the proposition should appear on the November ballot are the followers of Lyndon LaRouche, a right-wing political extremist. Brian Lantz, a spokesman for the Prevent AIDS Now Initiative Committee (PANIC), says his group filed a petition with 680,000 signatures to get Proposition 64 on the ballot. Lantz says people are worried that health officials do not have all the answers about AIDS, and are not doing enough to protect the public from the disease.

As an example of persisting uncertainties about the transmission of the AIDS virus (HIV), Lantz points to the cluster of cases in Belle Glade, Florida. So far, he says, there has been no adequate explanation why Belle Glade should have so many AIDS cases.

It is unclear exactly what health officials will be required to do if Proposition 64 passes. Certainly, more people will be subjected to antibody tests, and those found positive may be prevented from working in schools, health care facilities and food service occupations. Supporters of the measure say that, while it may not be mandatory that carriers of the virus should be put in quarantine, that avenue should be left open. Indeed, PANIC regards quarantine as an acceptable and possibly necessary option for controlling the spread of AIDS. The proposition would also require reporting of carrier status to state health officials.

Proposition 64 has provoked tremendous opposition from the Californian medical and public health communities. James Chin, chief of the infectious disease branch of the State Department of Health Services, calls the proposition "absurd", saying that public health officials are

already taking the necessary steps to control the spread of AIDS. He denies that there is uncertainty about the transmission of AIDS, and that although adding virus envelope may change with time, its infectivity is unlikely to undergo dramatic changes.

Chin does, however, admit the possibility that the virus could radically change in time, but says that should not be the basis for establishing public health policy. "You could play those 'what-if' games endlessly," says Chin.

Paul Volberding, chief of the AIDS activities division at San Francisco General Hospital, which pioneered the treatment of AIDS patients, also believes that the evidence that HIV is not a casually infectious agent is "overwhelming".

The reporting requirements of Proposition 64 are especially troubling to health officials. Mervyn Silverman, director of the AIDS health services programme at the University of California, San Francisco, worries that the social stigma of the disease and the possibility of quarantine will keep patients out of the health care system at the early stage of the disease when they could be helped. Lack of confidentiality about carrier status would dissuade people from voluntarily submitting to blood tests, making it harder to keep track of the spread of HIV.

AIDS in Japan

Test-kit market opens up

Tokyo

FROM this month, Japan's will screen all blood donors in Japan for acquired immune deficiency syndrome (AIDS) and adult T-cell leukaemia (ATL). The decision opens up a huge market for makers of antibody test kits with an estimated budget of ¥5,000 million (£22.5 million) for the screening programme. So far, 6 carriers of AIDS antibodies have been found among about 900,000 donors in the Tokyo and Osaka areas. The official number of AIDS cases in Japan has risen to 21, 13 of which have proved fatal.

There have been calls for mandatory AIDS and ATL screening of blood donors in Japan since last year when the first AIDS cases were reported and the dangers of ATL infection through blood transfusion became apparent (*Nature* 318, 306; 1985). But the Health and Welfare Ministry decided to introduce only partial screening earlier this year.

ATL seems to pose a more immediate threat to the nation's blood supplies than AIDS. More than 33,000 people with antibodies to the ATL retrovirus (HTLV-1) have been found among about 700,000

Opponents of Proposition 64 hope to raise \$8 million to defeat the initiative. Silverman agrees the proposition must be defeated, but is dismayed at having to spend money that would be more useful for research or education.

Most voters are opposed to the proposition. A poll by the *Los Angeles Times* published on 13 September showed 51 per cent opposed, 35 per cent for and 14 per cent undecided. A *San Francisco Examiner* poll in early September had 41 per cent opposed, 17 per cent for and 42 per cent undecided.

Even if Proposition 64 is defeated, unless it is lost by a large margin, Silverman says "we've got big troubles." Silverman worries that anything but a crushing defeat in California will encourage LaRouche supporters elsewhere to introduce similar propositions.

Among politicians, opposition to the proposition cuts across traditional political lines. Both Republican and Democrat candidates for Governor and Senator have opposed it, as does the director of the state's Department of Health Services. But Lantz says politicians opposed to the proposition are out of touch with the body politic. He believes that the voters in California are not satisfied with glib assurances that they have nothing to fear from AIDS. Opponents of the proposition agree that fear is indeed the issue. They hope to convince voters not to let their good sense be overcome by PANIC.

Joseph Palca

donors in Kyushu in southwestern Japan since the Red Cross began screening in February. And in several other parts of the country the occurrence of carriers exceeds 1 per cent. Although only about 1 in 2,000 carriers develop the disease, it is almost invariably fatal.

Japan's Red Cross will use enzyme-linked immunosorbent assay (ELISA) tests developed by Abbott Laboratories of the United States and by Organon of the Netherlands for AIDS screening. But other tests will be considered; one likely candidate is the gelatin particle agglutination method developed by Fuji Rebio of Japan.

The principle of Fuji Rebio's method is simple. Micrometre-sized particles of gelatin and arabic gum are coated with dead viral antigen. A suspension of the antigen-coated particles is then mixed with the test serum and allowed to settle. Clumping of the particles indicates the presence of antibodies. The method has already been successfully used for ATL screening and the company has applied for approval of its AIDS test based on the same method.

David Swinbanks

In vitro fertilization

French scientist makes a stand

JACQUES Testart, the leading French specialist in human *in vitro* fertilization, has committed "professional suicide". That is how he describes his decision to pull out of the race to develop the technique further, except insofar as it will aid infertile couples. Testart, whose group was responsible for the first French test-tube baby, and has since "midwived" 210 more, is sick of the "bébé spectacle" and worries about future "perversions" of the technique.

According to Testart, within a decade everyone will be demanding test-tube babies because it will be possible to determine the sex of the offspring, and to detect genetic and other abnormalities by dividing the early embryo and growing part of it for genetic sampling and tissue testing. Testart is not against these procedures to reduce genetic and sex-linked diseases, and he is pro-abortion ("it exists, and I have no objection in principle"), but he fears where they may lead: "I agree, but



I'm afraid: if we have such techniques we can use them for many things." Eugenics is not far away. "I think it's better to abandon the technique than to take the risk." The *in vitro* technique is beginning to create "new demands and new anxieties" rather than treat medical problems such as infertility, which should be its function.

In a book published this month (*L'oeuf Transparent* Flammarion, Paris, 1986) Testart opens with a quotation from the philosopher Kant: "make sure your maxims are those of which you could accept all the consequences". Ethics must come before research, says Testart. He condemns proposals to create banks of individually tailored spare parts, male pregnancies and human births from an animal womb or vice versa. He also fears the consequences of the possible fertilization of eggs by eggs (allowing homosexual or parthenogenetic births) and human cloning. In an interview, he separated himself and his col-

leagues from all such developments. "My group is no longer in the race for 'firsts'".

Testart last week was disappointed that he had had little reaction to his stand from scientists, and in particular none from the leading countries in the field, Britain, the United States and Australia. "I've had plenty of interest from journalists, sociologists and philosophers, but nothing from scientists" said Testart, "except for support from the leader of one French research group in Montpellier". From the other 8-10 research groups in *in vitro* fertilization in France, he has heard nothing.

His group, he says, is behind him but he admits the future of his laboratory "is a problem". He intends to continue research, but to restrict his work to the freezing of human eggs; to achieving successful fertilizations when the sperm is very poor; to understanding the maturation of the egg; and to anything directed to reducing a couple's infertility rather than creating new needs. But there must surely now be a question-mark over the position of Testart's laboratory in Professor Emile Papiernik's clinic at the Hôpital Antoine

Béclère, where Testart described Papiernik as "ambiguous" about the issues raised. **Robert Walgate**

- Last week the Council of Europe, a body which draws members from parliaments throughout Europe and with considerable influence, though no actual power, adopted a clutch of rules on the use of human embryos which the Council expects individual governments to adopt. They should please Jacques Testart (above). The rules would forbid the creation of human clones the implantation of embryos in the uterus of another species or the reverse; the cross-species fertilization of germ cells; the creation of embryos with the sperm of different individuals; the creation of chimaeric individuals; the bringing to term of an embryo outside the female uterus; the creation of individuals from parents of the same sex; the choice of sex of offspring, except for therapeutic reasons; the creation of identical twins; and any experiment on living embryos to fourteen days at normal temperatures (in other words, excluding time the embryo may spend frozen). The Council would also forbid the extraction of any tissue from an embryo unless beneficial to the embryo itself. □

Scientific collaboration

When the East meets the West

THE science division of the North Atlantic Treaty Organisation (NATO) last week hosted an international seminar on Soviet science. The idea of the Soviet science symposium was triggered by Lord Carington when he became head of NATO two years ago and who believed that the NATO science division needed to be better informed about science in the Soviet Union.

The seminar covered a wide range of topics from many scientific disciplines. What it revealed, however, was not so much gaps in western knowledge of Soviet science, but some profound differences in how the different academic disciplines view Soviet science effort.

Participants at the seminar were either scientists in the "hard" sciences, scientific administrators responsible for exchange programmes or Soviet analysts. Each group, it soon became clear, perceived Soviet science in different ways. To the analysts, for example, the structure of Soviet scientific institutions, each with its military department, responsible for relaying useful data to military establishments, is a basic datum, but several of the scientists and administrators expressed shock that the exchange programmes they sponsored or took part in could have a covert military dimension. A useful conclusion which was accepted by all groups was that it is somewhat misleading to lump

together "science and technology" in the planning of exchange programmes, although this has now become standard practice in international agreements. For although basic research is aimed at increasing and disseminating scientific knowledge, applied research, being aimed at the production sphere, inevitably involves some commercial secrecy.

The chief barriers to scientific cooperation with the Soviet Union seemed well-known to all participants: the difficulties of bringing a Soviet colleague to an international conference, the lack of available information about which Soviet laboratories are doing significant work in one's own field, or about the *curriculum vitae* of a Soviet scientist proposed for an exchange. On the Soviet side, there were of course the problems of access to western journals and the low standard of Soviet publications because of the lack of a peer review system, and a hierarchical research structure which (at least until Mr Gorbachev's reforms) fostered routine rather than imaginative research.

There was much curiosity about how far the Soviet Union had gone with creating the promised computerized database for the entire academy network and whether or not this will be linked to similar networks in the West, given the Soviet authorities' known emphasis on secrecy.

Vera Rich

Chernobyl

Pact for nuclear accidents

FIFTY-ONE out of the 113 member countries of the International Atomic Energy Agency (IAEA) last week signed two conventions on the rapid notification of nuclear accidents and on mutual aid between neighbouring states in the case of a nuclear accident. First to sign was the Soviet Union, followed by West Germany, the United States, East Germany and China. There were few surprises; Jordan and Ghana did not sign while Luxembourg, which was intending to sign only the notification convention, had still not done so by Monday this week. Soviet Ukraine and Byelorussia, the republics most affected by Chernobyl, signed separately.

The two conventions had been on the IAEA agenda since 1982, but before the Chernobyl accident in April 1986 little progress was made. Since Chernobyl, the Soviet media has claimed the idea of the conventions as a Soviet initiative, and, two days before the documents were opened for signature, the TASS news agency announced a "programme for creating an international system of safe development of nuclear energy".

The programme mainly consists of the two new conventions plus several of the recommendations of the Chernobyl post-accident review conference in Vienna in August. These include rapid notification procedures, procedures for dealing with nuclear accidents, all countries to abide by the IAEA safety recommendations, exchange of information on accidents and near-misses, the joint development of fusion power and the protection of nuclear power stations from terrorist action.

More interesting is the Soviet stance on civil liability for nuclear damage. According to the proposed programme, attempts at "international legal regulation" in this field have so far not been "sufficiently worked out". In fact, the three Soviet United Nations delegations took part in the drafting of such a convention in the early 1970s, but failed to sign it. But, the Soviet programme suggests, a possible future liability agreement might provide for states to be responsible not only for the damage caused by cross-border radiation following an accident, but also for "material and moral-political damage" due to "unjustified actions taken on the pretext of protection from the consequences of nuclear accidents". Spreading "unscrupulous reports" and introducing "unjustified restrictive measures" — the two charges the Soviet media made against the West after the Chernobyl accident — are cited as the type of action for which the country where the accident took place could demand compensation.

Vera Rich



FINAL tests were underway this week in anticipation of a non-stop around-the-world flight by Voyager, the unusual looking aircraft pictured above. If all goes according to plan, Voyager will take off from the Mojave desert at the end of this week, taking a great circle route around the Earth, returning to the California desert in 10 to 12.5 days. Voyager was designed expressly for the round-the-world flight. At take-off it weighs approximately 9,300 lbs, of which 7,000 lbs is fuel carried in 15 separate tanks. The flight path will take Voyager over southern Africa and Northern Australia, but 95 per cent of the flight will be over water. US satellites will help track the aircraft and its two-person crew. Voyager has a wingspan of 110.8 feet, a fuselage length of 25.4 feet and a vertical tail height of 10.3 feet. The exterior surface of the plane is made of Magnamite, a composite graphite material.

Joseph Palca

French science

More for defence; less for science

THE French government is to spend 20 per cent more on defence research than its predecessor, a leap which offsets falls in other sectors of government-sponsored research to produce a net rise in research spending of 8 per cent in 1987, according to the full government budget announced late last month. But these figures conceal a sorer story for basic science.

Earlier figures discussed in these pages (31 July p.400 and 4 September p.3) are confirmed in the full budget. They represent a fall from the previous government's planned spending in 1986 on basic research of some 5 per cent, bringing 1987 spending levels to around FF 21,000 million (£2,100 million) including salaries, which is about what French scientists enjoyed in 1985. However the research ministry (now the ministry of research and higher education) now represents essentially only basic and strategic applied research, and the budget of all ministries now reveals the increased spending on defence research and development, which will rise FF 5,000 million to nearly FF 31,000 million in 1987. The principal target of this spending is the modernization of the French nuclear force, particularly land-based missiles. The defence ministry receives an increase of more than 13 per cent in capital spending in the 1987

budget.

Some crumbs of comfort for university and research council laboratories are present in the new budget, however, with FF 60 million of the education budget allocated for improving France's poor position in university computing. However, the expected decline in direct government support of industrial research is confirmed. The government has previously berated French industry for its poor commitment to research (private spending accounts for 43 per cent of research and development, against 49 per cent in the United States) but believes that direct, programmed support from government is misguided. Any more aid will come in the form of tax breaks for research expenditure than in direct programme aid, but the total support is expected to be down, as industry will inevitably take time to respond to the incentives.

Government spending on space will increase 5 per cent to FF 4,400 million. This is not enough to impress the previous president of the French national space agency and the last minister of research under the previous government, M. Hubert Curien, who says the reductions in support of industrial research would penalize the regions, where applied research was concentrated in small companies.

Robert Walgate

Biotechnology

New regulations in dispute

Washington

JUDGING from criticisms aired last week, the US government's coordinated framework for biotechnology regulation may not be so coordinated after all. Comments from several trade groups suggest that the framework has failed to clarify agency jurisdiction and has cast the regulatory net too widely. The results could be cost and confusion for an industry dominated by small companies with little spare change.

The Industrial Biotechnology Association (IBA) and the Association of Biotechnology Companies (ABC), both major advocates of commercial biotechnology, voiced their concerns in their formal responses to the framework, published in June under the aegis of the Office of Science and Technology Policy (see *Nature* 321, 458; 1986). The document includes definitions of organisms subject to federal review and statements of policy from the three agencies involved — the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA).

There have been many detailed complaints since then, but critics are dissatisfied with the overall picture. The framework's primary purpose was to spell out the regulatory interactions between the three federal agencies. Yet IBA, ABC and the Pharmaceutical Manufacturers Association have all pointed to overlapping and uncertain areas of responsibility between the EPA and the USDA.

The framework suggests that one agency be designated the lead agency with primary responsibility, while the other is considered the secondary agency. But it does not elaborate on the role of the secondary agency and thus could lead to conflicting interpretations without avoiding duplication.

ABC notes that a Cornell University researcher has already become entangled in the regulatory web. Gary Harman, at the New York State Agricultural Experiment Station, requested permission last spring to test, in the field, a recombinant *Trichoderma* fungus created by protoplast fusion. EPA conducted the extensive review required to grant an experimental use permit, then decided last month that the organism did not need one.

In the midst of EPA's review the Animal and Plant Health Inspection Service, a branch of the USDA, was notified of Harman's intentions and began its own review. Despite the thorough scrutiny by the EPA, the USDA asked for additional data. And despite the EPA's earlier decision, USDA says that allowing Harman to proceed without further review would violate its statutes.

Within the vagaries of jurisdiction, the trade associations have also found several proposed definitions unpalatable. The definition of "pathogen" has roused the objections of the IBA because it includes microorganisms that have inserted genetic material from known pathogens, regardless of whether the inserts are involved in pathogenic mechanisms. The association wants several exemptions based on the likelihood of conferring pathogenicity with the transferred sequences. Such modifications in definition would not remove the recombinant organism from regulatory consideration, but would ease a stiff review.

Intimately related to the issue of reviewing organisms for environmental release is the determination of exactly what constitutes environmental release. Both IBA and ABC feel that no consensus has been achieved within the industry or among the agencies to arrive at a general

definition. Likewise, the issue of containment, which can carry enormous financial demands, has yet to be hammered out. The EPA has expressed willingness to loosen monitoring of recombinant organisms used only in closed systems such as fermentation vessels; but the characterization of a closed system turns on containment standards.

The trade associations also used their comments on the framework to frame their less technical criticisms. EPA is commended for its shift from process-based to product-based policy, a shift urged by the industry in 1984.

Despite the conflicts surrounding regulatory policy, present tensions may well dissipate as interpretation takes the place of speculation. FDA, the agency most conversant with recombinant DNA regulation, issued the tersest policy statement and received the warmest remarks. The industry has appreciated FDA's case-by-case approach. But as the number of recombinant products mounts, the agency may no longer be able to take the time for individual attention.

Karen Wright

Chernobyl fallout

Pugwash's radioactive trousers

A LETTER by scientists from the University of Groningen is published on page 399 of this issue. The group has been investigating core fragments from Chernobyl "inadvertently" picked up by travellers returning from the Soviet Union.

A colourful and anecdotal account of this work was given last month at the 36th Pugwash conference on Science and World Affairs in Budapest. Dr Phillip Smith, a physicist at Groningen, described how a Dutch student, returning from Kiev shortly after the accident, brought in his

risk factor is two orders of magnitude less.

That Pugwash should be discussing the Chernobyl fallout at all represented a new departure. The hazard of fallout was discussed by the first Pugwash Conference almost 30 years ago, but then it was the fallout from nuclear tests which posed the main risk.

It was Dr Joseph Rotblat of London who suggested some months ago that since, after all, Pugwash was a gathering of scientists there should be at least one scientific lecture, and after Chernobyl, the issue of reactor safety seemed an obvious choice. In the event, the information content of the formal presentations was marred by the fact that the long-term projections of the effect of fallout in Europe had been based on British and Dutch computer models which had assumed that the emissions had lasted hours rather than (as the Soviet report to the International Atomic Energy Authority revealed) two weeks.

But the idea of using a non-confrontational forum such as Pugwash to air issues of international public concern is an interesting one, which if repeated could well add a new and valuable dimension to the work of the Pugwash movement. Unfortunately, the final statement from the conference, which concentrated on the major issues of a comprehensive test-ban, the reduction of East-West tensions and the destabilizing effects of the debts of developing countries, made no mention of any plans to repeat Dr Rotblat's initiative.

Vera Rich



trousers for screening, and how they had revealed "hot" particles of plutonium, americium and curium. The Soviet delegates to Pugwash did not contradict this account, although Academician Vitalii Goldanskii contended that Dr Smith was being unnecessarily alarmist in mentioning plutonium. The "hotspots" he said, were predominantly curium, for which the

Voyager

Looking ahead to Neptune

Pasadena

VOYAGER 2, now old and far away, has nonetheless given the National Aeronautics and Space Administration (NASA) one bright spot in an otherwise gloomy year. So, on 19 September, NASA said thank you by handing out a spate of awards to Voyager team members, including the agency's highest award, the Distinguished Service Medal, to Richard Laeser, Voyager's project manager since 1981.

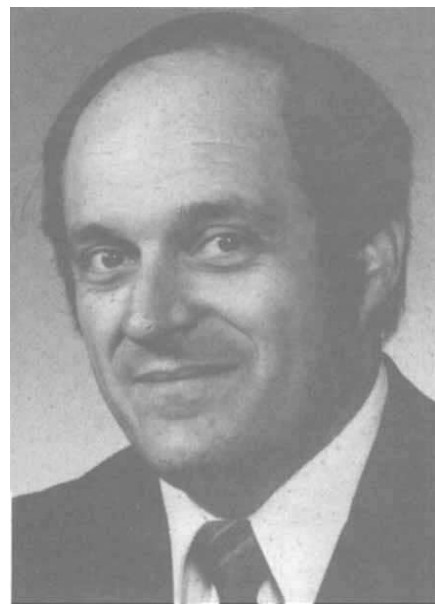
Under Laeser's direction, Voyager 2 was essentially re-engineered from the ground while on its way from Saturn to Uranus. The result was a much more fruitful encounter than anyone could have predicted when the spacecraft left Earth in 1977. Now Voyager team members are looking towards the encounter with Neptune in 1989, and in the next few months Laeser and his colleagues at the Jet Propulsion Laboratory (JPL) face some tough

decisions about the character of this next planetary visit.

The main issue that Voyager's planners are wrestling with concerns the spacecraft's trajectory. A close approach is best for imaging, but makes problems for radio science. If Voyager passes too close to Neptune, it could experience atmospheric drag, something it is not built to withstand. Even a small amount of drag could affect the spacecraft's attitude.

Another difficulty in too close an approach to Neptune is the possibility of an unintentional encounter with one of Neptune's recently discovered fragmentary rings. That, says project scientist Edward Stone, is unlikely, since the ring arcs account for only about 10 per cent of an entire ring, and are quite narrow. Even hitting them intentionally would not be easy.

The decision about how close to come to Neptune will have a profound effect on



Laeser: re-engineered Voyager between planets.

how near the spacecraft will approach Neptune's large moon Triton. Measuring from the centre of the planet, a flyby of Neptune at 27,500 km would bring Voyager as close as 25,000 km from the centre of Triton. But a Neptune flyby at 32,300 km puts Voyager 68,000 km from Triton. The advantage of a more distant approach is that there would be more margin for error as the spacecraft tracks the moon's limb with its radio antennas.

The radio occultation experiment will provide details of the planet's atmosphere. At Triton, a far approach also gives a benefit to the radio science team, but complicates the timing of a second experiment using scattered ultraviolet light from a solar occultation. A close approach to Triton would not only give better picture opportunities, but also allow an infrared investigation of Triton's atmosphere. A similar experiment earlier in the mission discovered hydrocarbons in the atmosphere of Saturn's moon Titan.

Laeser says that a decision on the trajectory will be made in the next few months. The sequence of events at the encounter will be planned on a second-to-second basis as the spacecraft makes its closest approach to the planet and moon.

The Voyager team now consists of 160 people at the Jet Propulsion Laboratory, down from a peak of 200 earlier in the year during the Uranus encounter. Laeser expects that number to stay fairly stable until just before the Neptune encounter.

Despite NASA's budgetary uncertainties, Laeser expects Voyager to get the funds it needs to achieve all its objectives at Neptune. Laeser's confidence comes from the programme's good track record of sticking to its budget. Besides, he believes NASA is unlikely to do anything that will dim the brightest star in its currently murky firmament. **Joseph Palca**

Deltaplan defence line completed

Waalre, The Netherlands

On the night of 1 February 1953 more than 1,800 people lost their lives when the North Sea breached the Dutch sea defences, flooding large areas of south-western Holland. To stop such a disaster recurring, a remarkable civil engineering project — the Deltaplan — was drawn up, and the last link in the defensive chain is about to be officially opened.

The overall effect of the Deltaplan is to reduce the exposed coastline of Holland by some 700 km. The last link, a storm surge barrier across the Eastern Scheldt estuary, is the biggest single project in the plan. Not only is it the most costly project, but also the most controversial. The original plan had involved a closed dam across the 9-km-

wide estuary, but pressure from conservation groups and the fisheries industry led to the change to a 3-km-wide storm-surge barrier that allows the tide to flow normally but permits the closure of the estuary if floods are predicted.

In its final form the barrier is made up of 66 piers placed in three tidal channels; four times a day 800 million cubic metres of water passes through the 63 gates in the barrier. Model studies suggest that there will be considerable changes in the ecology of the area if the barrier has to be closed. Even without closures, there are worries about the sensitive oyster beds in the east of the estuary, where the mean tidal range will be reduced from 3.5 to 2.7 metres. **Casper Schuurin**



Some of the 63 gates in the Eastern Scheldt barrier. A public road runs along the top of the barrier, 12 metres above sea level.

Research integration

If you go to San Francisco . . .

San Francisco

SEEKING to sustain the position of the University of California San Francisco (UCSF) Medical School as a premiere biomedical research institution, a group of faculty members has put together a new programme that they hope will "both exploit and advance the revolution in biological research." With good prospects for funding and the promise of precious laboratory space from the medical school administration, the new Program in Biological Sciences (PIBS) may well supplant traditional departments in guiding graduate education and research.

As initially implemented, the new regime will involve a confederation of four graduate programmes; biochemistry, cell biology, genetics and neuroscience. While each member department will retain its individual identity, PIBS will admit graduate students, advise on new appointments, generate funds and foster cooperation among different disciplines. Guiding PIBS will be a board of faculty members, two from each of the member programmes, plus J. Michael Bishop who was acting as board chairman until he went on sabbatical at the beginning of this month.

To work, PIBS must effectively coordinate interdisciplinary research. But that should not be a problem, says PIBS board member James Hudspeth, since cooperation has always been a strong suit of the medical school faculty. For nearly 50 years, following the great earthquake in 1906, San Francisco's basic research faculty was exiled to the University of California Berkeley campus across the bay. It was a relatively young and enthusiastic faculty

that returned to San Francisco in the 1960s and 1970s, bringing UCSF its current level of success. One measure of that success, boasts Dean Rudi Schmid, is that UCSF now receives about \$80 million in support from the National Institutes of Health, more than any other single institution.

For Bishop, the wide spectrum of faculty interests is what will make PIBS work. "Nothing succeeds like diversity", he says. He argues that traditional medical school departments are anachronisms, since modern biologists draw on several disciplines in their work.

While membership in the new programme is at present limited to just four departments, expansion is inevitable. One goal of the new programme, says Hudspeth, is to prevent any one department from getting richer and "building a wall around itself". Biochemistry has been the most



Diversity succeeds, says J. Michael Bishop.

successful department financially at the medical school, and there are those that worry that PIBS will merely become a vehicle for biochemistry's greater glory. But Bishop disagrees, arguing that by becoming equal partners with other departments, biochemistry has diluted its potential influence.

While officials at UCSF would like to claim that careful planning and administrative acumen is solely responsible for the cooperative spirit and academic success that the university enjoys, they cannot. Says Dean Schmid with a smile, the key factor was luck.

Joseph Palca

Oceans and atmosphere

Advisory group sinks

Washington

It looks like the end of the road for the National Advisory Committee on Oceans and Atmosphere. The 18-member committee, formed in 1975 to give the private sector an advisory role in ocean and atmosphere policy, is likely to disband on 1 October, and there seem to be few who will mourn its passing.

The committee, chaired by John Flipse of Texas A&M University, was originally conceived as a means of giving the private sector a voice in policy advice; its members include representatives of academia and industry. But on Capitol Hill there is a growing feeling that the committee has become politicized and that it lacks the "expert" status it once had. And as it is staffed by employees of the National Oceanic and Atmospheric Administration, it lacks sufficient independence to give good advice.

The committee has recently concentrated on providing recommendations on the US Exclusive Economic Zone announced by President Reagan in 1983. What will probably be its last opus is an assessment of the National Oceanic and Atmospheric Administration. But the administration feels the committee is giving it advice it does not need, and has been trying to abolish it for several years.

Congress has so far always stepped in at the last moment with a reprieve, but this year even Congress's patience with the committee seems to have been exhausted. A new bill has been introduced to establish a national marine policy commission, a blue-ribbon expert panel whose members would, unlike the committee, be nominated from a list provided by Congress. Among the issues the new commission would tackle are international marine policy, conservation of marine resources and establishment of proper roles for federal and state governments and the private sector in marine policy.

Tim Beardsley

Mutation screening an increasing factor

Washington

CONGRESS's Office of Technology Assessment (OTA) has provided a timely warning that new techniques for measuring human mutation rates will raise novel policy questions as their use becomes more widespread. The research office concludes* that within the next 5 or 10 years several new technologies for studying prevalence of mutations could be ready for large-scale epidemiological screening of populations at risk, such as those exposed to mutagenic chemicals or anticancer drugs.

The new techniques, such as analysis of restriction-fragment-length polymorphisms, electrophoresis of protein variants and DNA sequencing, have the potential not only to screen individuals for recessive genetic diseases, but also to assess populations for exposure to mutagenic influences such as some chemicals or radiation. Congress has already passed environmental

health laws that establish the public's right to avoid mutagens, but because of the obvious problems of using humans, experimental animals are used to establish mutagenicity. But all that could change if the promise of the new techniques is fulfilled.

OTA estimates that \$14.3 million was spent on human mutation research in the United States in 1985, most of it by the Department of Energy; the department's interest in the area dates back to studies of the Japanese atomic bomb blasts at the end of the Second World War. But field studies that would be necessary to verify large-scale tests could cost tens of millions of dollars. OTA recommends that Congress make the department "lead agency" for mutation research and establish a centralized samples bank for studying heritable and somatic variations.

Tim Beardsley

*Technologies for Detecting Heritable Mutations in Human Beings OTA, Washington DC, 1986.

In the footsteps of dinosaurs?

SIR—It is impossible to work on dinosaur footprints in Texas^{1,2}, without becoming involved in the “man track” controversy. I have seen most of the alleged human ichnites, but have been reluctant to publish interpretations without a thorough understanding of the details of formation of unquestioned dinosaur tracks, and the gaits of the trackmakers. Now Kuban and Hastings have sparked a revival of interest in this topic with their publications on the “colour distinction” of the Paluxy River “man tracks”^{3,4}. I am prompted to alter my approach to this matter by John D. Morris’s recent letter to *Nature*⁵.

Most of the structures identified as “man tracks” in Lower Cretaceous rocks at Glen Rose and elsewhere in Texas are not footprints of any kind, let alone human tracks, but are simply solution features and/or scour marks of the kind that can be seen in riverbeds throughout central Texas, and even on exposed rock surfaces well away from river valley. Some of the “man tracks” do occur in good trackways, however, and clearly represent the footprints of bipedal animals. The Taylor trackway, featured in much of the creationist literature, is a good example, and I have examined a similar trail in Hondo Creek, Bandera County. The footprints in these trackways are generally elongated depressions rather lacking in detailed morphology. If one looks at enough bipedal dinosaur footprints, at enough sites in Texas, it is possible to find footprints intermediate in shape between the elongate depressions of the “human” trackways and more normal tridactyl footprints of typical bipedal dinosaur trails.

I have measured stride, pace and footprint lengths of over 70 bipedal dinosaur trails in Texas, and supplemented these data with measurements from the literature of more than 650 additional bipedal dinosaur trails²; the “human” trails match typical dinosaur trails in the relationship between footprint size and pace or stride length, and also show similar step angles. Thus even before the “colour distinctions” were recognized, there was ample reason for regarding the Paluxy River “human” trackways as the poor end of the impression/preservation spectrum of theropod or ornithomimid trails^{6,7}.

Kuban corroborates this interpretation by making a good case⁴ that many of the “humanoid” footprints were formed by bipedal dinosaurs walking in an unusual, “flat-footed” fashion, with their metatarsals pressed against the substrate. Similar metatarsal tracks have been reported from a Cretaceous site in Queensland⁸, and perhaps elsewhere. If the toe marks of such footprints were to collapse, as some-

time happens during footprint formation, very human-like tracks would be formed. In the case of the Taylor trackway, however, the toe impressions apparently did not collapse. Weathering of the rock surface has resulted in the development of more or less non-impressed or irregularly impressed, coloured toe marks and a larger track outline associated with the more deeply impressed, originally discovered “man tracks”⁴. Although unusual, the phenomenon is not “unprecedented,” as Morrison states; an ornithomimid trail with footprints delimited mainly by such colour distinctions occurs in the Dakota Group (Cretaceous) along the Alameda Parkway near Denver, and some of the sauropod footprints from the Purgatory River (Morrison Formation, Jurassic) of Colorado are distinguishable from the surrounding rock mainly by their colour (M.G. Lockley, personal communication).

The origin of such colour-delimited footprints was much discussed by participants on the field trip of the recent First International Symposium on Dinosaur Tracks and Traces (Albuquerque, May 1986). The tracks were perhaps filled with sediment of a contrasting type from that in which the footprints were impressed; this inhomogeneity may have led to the colour distinctions in response to modern weathering. Alternatively, as dinosaurs crossed mud flats, their weight may have squeezed pore water from the sediments beneath their feet, resulting in subtle chemical differences from the surrounding sediments; the coloured tracks may thus have formed as “ghost tracks” some distance below the sediment/water interface.

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Greenhouse cure

SIR—Those who are concerned about the consequences of the “greenhouse effect” overlook the benefits that would follow from the general adoption of a vegetarian diet. If there were a halt to the consumption of grain-fed livestock products (while retaining the present pasture lands to support animals for meat and milk), at a per capita grain consumption of 200 kg per

year for a healthy vegetarian diet, only about 60 per cent of the present land under cultivation would be needed to feed the world population. With the further modernization of techniques in developing countries, that 60 per cent would be more than sufficient to feed the projected world population in the next century. This would not only stop the deforestation that contributes substantially to the increase in atmospheric CO₂, but would enable us to reforest 40 per cent of the present agricultural land, leading to large-scale absorption of atmospheric CO₂. Such an extensive mopping-up of carbon dioxide by young growing forests may be the only way to prevent the worrying consequences of the greenhouse effect, at least until alternative energy sources can effectively replace fossil fuels.

Legislation to effect a change in our dietary habits may be difficult to achieve. But fortunately, it is a step that we could fruitfully take at the individual level without having to wait for government action, the laws of supply and demand would go a long way towards making sure that reduced meat demands would decrease the pressure on land and pave the way for rapid afforestation.

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Damadian defended

SIR—It is disturbing that you should publish such a prejudiced review (*Nature* **320**, 318; 1986) of Sonny Kleinfeld’s book, *A Machine Called Indomitable*, containing derogatory remarks about Raymond Damadian, whose struggles have given the world a powerful tool for medical diagnosis. The reference to Damadian as a “misfit” does harm to a journal with a reputation for even-handedness and fair play. In his review Peter Newmark seems to take popular acceptance as the sole criterion for scientific excellence. If this were true, Copernicus, Lavoisier, Darwin, Planck and Einstein were all “misfits”, rejected by their contemporary peers.

Newmark’s comment that I, though successful in casting a spell over Damadian, nevertheless “remain a voice in the wilderness”, is also uncalled for. I suggest he reads the review (*Nature* **311**, 682; 1984) of my book, *In Search of the Physical Basis of Life*, where the reviewer, Dick, though a long-time scientific opponent of mine, stated with fairness and general accuracy: “Since the 1950s majority views of the cell have moved significantly nearer to those of Dr Ling...”.

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Making molecules into atoms

If the hydrogen molecule is two protons and one electron, how should it resemble the helium atom, which has one proton and two electrons?

THE hydrogen molecule-ion is of course the simplest molecule of all, which is no doubt why it has featured in elementary textbooks of quantum mechanics since virtually the beginning. Having dealt with the hydrogen atom, and shown that wave mechanics or some other calculating scheme can be made to work, textbook writers are naturally eager to show that the scheme will also work for molecules.

To be fair, the example of the hydrogen molecule-ion is as instructive as anybody could ask. One distinctive feature of this simple system is that it is a three-body problem which can no more be exactly solved in quantum than in classical mechanics. Another is that two of the three particles have equal mass. So, it may be asked, to what extent does the familiar treatment of the problem of the hydrogen molecule-ion apply to that other elementary three-body problem with two out of three masses equal, that of the helium atom?

This question has now been taken up by James M. Feagin of the California State University at Fullerton, and John S. Briggs from Freiburg University, West Germany (*Phys. Rev. Lett.* **57**, 984; 1986). On the face of things, it is a wrong-headed question. The unthinking assumption of quantum chemistry is that the motions of massive atomic nuclei are necessarily slow compared with those of the electrons in a molecule, and so may be neglected while calculating the patterns of electron distribution. This is the Born–Oppenheimer approximation, whose validity is attested by the fact that electronic transitions usually yield spectral lines in the visible or ultraviolet while nuclear motions (vibrations) yield spectral lines in the infrared.

A little thought, however, will show that the truth is not quite as simple, and that there is an uncomfortable element of circularity in anything that pretends to be a complete treatment of even the hydrogen molecule-ion. Suppose, for example, that the objective is to calculate not simply the allowable electronic states but the vibrational and rotational energy levels as well. The Born–Oppenheimer approximation first requires the calculation of the electronic states, each of which then determines the potential in which the two nuclei vibrate. For each electronic state, there will be an infinity of vibrational states. And for each combination of electronic and vibrational states, there will be an infinity of rotational states.

What the elementary books on quan-

tum chemistry fail to explain is that empirical spectroscopic evidence that might test these calculations is thin. Moreover, such tests as there may be tend to disappoint the calculators. But nobody need feel ashamed, for the complete calculation of the hydrogen molecule-ion, rotations as well as vibrations, depends on a succession of manifestly false assumptions. So, too, must any attempt to calculate the properties of a helium atom by the techniques worked out for the simplest molecule ion. But if the numbers are precise, their patterns could be instructive.

This is the line that Feagin and Briggs have followed. The argument is impeccable. In the usual calculation of the helium atom (two electrons and a more massive nucleus held together by Coulomb forces), a decent starting point is to suppose that each electron moves as if in a hydrogen atom, possibly with a central electric charge different from one (hydrogen) or two (helium) but somewhere in between. Then, the recipe says, take products of these wave functions (one for each electron) as starting points, and find which linear combinations of these products best suit the case.

Obviously, if the inter-electron distance in a helium atom is to correspond with the inter-proton distance in the calculation of the hydrogen molecule-ion, there will be a sense in which electronic states of the helium atom correspond to vibrational states of the hydrogen molecule-ion. Similarly, rotational states of one problem will correspond to vibrational states of the other. But the numbers calculated for, say, energy-levels will be very different. Only the countable triple infinity of states, and their symmetries, will be recognizable.

Nobody should mistake what Feagin and Briggs have done for a way of calculating the properties of helium atoms (or even those of the negative ion of positronium, one positron and two electrons). But it is vividly illustrative of several intriguing problems, especially of the molecular orbital approach to molecular calculations.

There, as the textbooks explain, the classical treatment of the hydrogen molecule-ion begins by supposing that one of the protons is very distant from the other. Then, to a very good approximation, the state of the system is that in which the electron is attached to either one or the other of the two protons or, naturally,

is some linear combination of the two. By allowing the distance between the two protons to shrink gradually, it is then possible to enumerate (but not to calculate exactly) the energy states of the molecule-ion in which the two nuclei are not widely separated from each other. In the prehistory of the 1930s, in the hands of people such as R.S. Mulliken, arguments like these made possible the extension of spectroscopy from atoms to molecules.

Feagin and Briggs attempt something along these lines. They do not abandon the advantage of knowing that nuclei are more massive than electrons, and therefore relatively immobile, but rather suppose that one point in the triangular interaction (the helium nucleus) is fixed in space. The hierarchy of quantum numbers at which they arrive is a shuffled version of those arising in the usual calculation of the helium atom: first comes a number corresponding to states of inter-electronic separation (electronic vibrations relative to each other), then one determined by both interelectronic separation and that of the electronic centre of gravity from the nucleus (total angular momentum?) and, finally, one involving the full geometrical coordinates of the three particles.

The outcome is impressive. Simply by scaling previous results for the hydrogen molecule-ion to allow for the differences of mass and charge, Feagin and Briggs are able to produce curves which show the energy of states of the helium atom as a function of inter-electronic separation whose symmetry is also well-defined. The description may be unfamiliar; those elementary textbooks tend to assert that inter-electronic distances are meaningless, which is true only to the extent that the Born–Oppenheimer approximation gives greater meaning to the concept of inter-nuclear distances.

The more general implication of this arresting piece of work is that it emphasizes, yet again, that quantum mechanics is just as positivist in its methods as was classical mechanics before it. If you have an arbitrary mechanical system, it is a matter of free choice how coordinates are specified for its description. Broadly speaking, the choice does not matter. But prudent physicists try to find coordinates with physical meaning, coordinates corresponding to measurable quantities. Even more prudent people try several choices, knowing that one may illuminate another.

John Maddox

Earth sciences

Deep reflections on the Moho

from Penny Barton

PARTICIPANTS left a recent meeting* with the uneasy feeling that the Mohorovicic discontinuity (the Moho), which marks the base of the Earth's crust, is no longer the immutable feature of textbook tectonics. Hitherto the Moho, which lies around seven kilometres deep under the ocean floor and an average 35 kilometres under the continents, has been treated as a passive marker which may be displaced by tectonic events or isostatic adjustment, but generally stays where it is put. The meeting raised questions about the Moho's stability in time, its memory of previous history, its identification from a number of similar features at any one location and, indeed, its nature.

In 1984, the first meeting on deep reflection profiling of the continents formed

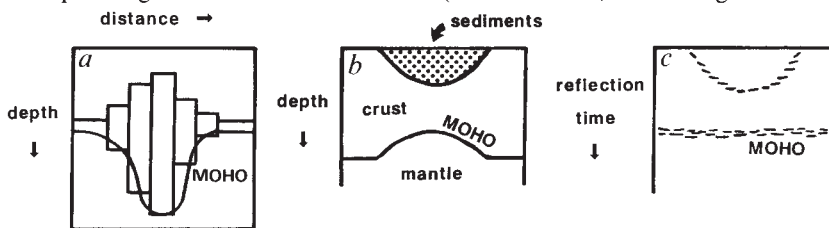
mentary basins at the top of the section: this implies topography in depth and Airy-type isostatic compensation at the Moho (see figure). However, new data show that the reflection Moho is sometimes neither flat in time nor depth (S. McGeary, Cambridge University) whereas other reflection Mohos are flat in depth irrespective of surface complexity, implying 'healing' of the Moho with time (L. Brown, K. Nelson, C. Potter, Cornell University; R. Meissner, Kiel University; M. Cazes, Elf-Aquitaine, Paris). Such Mohos have no obvious isostatic significance. Attention was drawn to the fact that the Moho is flat under old (more than about 300 million-year-old) mountain belts, rather than showing the conventional mountain root (D. Matthews, Cambridge University;

reflection Moho is established in new crust only 100,000 years after its creation at the Juan de Fuca ridge (R. Clowes, British Columbia University).

What are we looking at down there? Arguments continue about what gives rise to the lower crustal reflections, and it is as difficult to explain the common lack of reflectors in the upper crust as to explain their predominance in the lower crust. The field is divided broadly into the geological-structuralists versus the physical-propertyists. The structuralists show excellent modelling of both wide-angle and normal incidence reflection patterns using a model of alternating high and low velocity lamellae, perhaps sills, with thickness approximately 100 metres (G. Gajewski, F. Wenzel, E. Luschen, Karlsruhe University; D. Fountain, Wyoming University). The reflecting lower crust correlates with high refraction velocities and is more prominent in areas that have been extended (Mooney). The propertyists show a correlation between the depth to the top of the layering and surface heat flow, and hence with the 400°C isotherm (Klemperer). Electromagnetic studies show a highly conducting layer in the lower crust that could be explained most easily by the presence of free water from dehydration reactions; such water could form connected bodies at temperatures between 400 and 500°C (A. Jones, GSC Ottawa). The lower crustal layering never approaches a free edge, either (and most frustratingly) the Earth's surface, or, and perhaps significantly, the continental margin (Matthews, R. Hobbs; S. Fanavoll, IKU Trondheim). The structuralists would expect a Moho slow to move and difficult to eradicate; the propertyists predict something mobile in time and space, leaving no trace behind.

Some of the inconsistencies could be explained if both camps have part of the story. Intrusions into the base of the crust from the mantle could produce the bright, thin Moho reflector, whereas the thicker band of lower crustal reflections may be better explained by physical properties, which may not necessarily coincide with the base of the crust. Sometimes the two effects would be superimposed and the signals impossible to separate. Conversely the presence of one effect may be interpreted mistakenly in terms of the other.

The difficulty in interpreting seismic signals in terms of geology is a problem central to the subject. Seismologists often joke that one person's signal is to everyone else either noise or an artefact, but such misgivings are ever present. Several people at the meeting addressed this problem, of particular interest being a demonstration of the ability to distinguish between reflections from a thin layer of contrasting rock within a uniform matrix (a 'spike' in seismic properties) and an interface between two types of rock (a 'step') by looking at the change in frequency con-



Airy isostasy, *a*, Crustal columns of equal density float on the denser substratum: mountains are supported by 'roots', depressing the Moho. *b*, Low density sedimentary basins correspondingly produce 'antiroots', raising the Moho. *c*, The time taken for reflections from the Moho to return to the surface is increased by the presence of a low-velocity sedimentary basin causing 'velocity pull-down' on the time section which has the effect of flattening the Moho reflections if Airy-type isostatic compensation occurs at the Moho.

a consensus that more deep reflection profiles coincident with wide-angle seismic lines (conventionally called 'refraction' experiments) were needed to calibrate the spectacular features seen on deep reflection profiles against variations in seismic velocity. The growth of the field since then has been dramatic. Several high-quality coincident datasets now show a clear correlation between the refraction-defined Moho and the base of a reflective layer seen on normal incidence profiles, which varies between a single bright reflector and a reflecting zone of thickness five or six seconds two-way-time (W. Mooney, USGS Menlo Park; D. Stewart, USGS Reston; C. Powell, Cambridge University). Using this correlation, the Moho is widely identified on reflection profiles where there is no detailed refraction control. This is a useful working hypothesis, but strange things begin to emerge when it is applied in detail.

As a first approximation the time taken for reflections to return to the surface from the Moho is almost constant regardless of features such as low-velocity sedi-

Meissner). Data from the Georgia coastal plain and the US Cordillera show slanting reflections in the lower crust truncated against a flat reflecting Moho (Nelson, Potter): according to classic geological principles this implies a Moho as young or younger than the lower crustal structure. Some suggest the Moho could form a horizontal detachment between crust and mantle (Brown). There are several examples of the reflection Moho offset in both time and depth by vertical fault zones known to have major strike-slip component at the surface (McGeary; C. Keen, GSC Nova Scotia; P. Choukroune, Rennes University): strike-slip faults are perhaps unique in being able to juxtapose crustal columns of different thicknesses.

Mohos are springing up everywhere, sometimes several at a time. On some profiles there are several reflection Moho candidates, only one of which coincides with the refraction Moho (Mooney; McGeary). There are instances of closely spaced double horizontal reflection Mohos (S. Klemperer, Cambridge University; McGeary) and relic Mohos left behind during major tectonic events (L. Serpa, New Orleans University; R. White, Cambridge University). The oceanic

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tent with offset (J. Louie, California Institute of Technology). Normal incidence reflections arise from contrasts in acoustic impedance (seismic velocity times density) occurring over wavelengths short relative to seismic wavelengths. If we are lucky we can measure the seismic velocity and guess the density. However, even if we are able to determine the acoustic impedance of a lower crustal rock, its petrology is not uniquely defined. Thus it is vital that use is made of every other piece of information available. New wide-angle datasets using shear waves as well as compressional waves show that shear-wave structure, and hence variations in Poisson's ratio, is another measurable property of the lower crust (A. Hirn, IPG Paris; R. Meyer, Wisconsin University; Wenzel, Gajewski; C. Wright, BMR Canberra). Intriguing correlations between reflectivity and high conductivity still emerge from the new generation of electromagnetic sounding measurements (Jones).

There is no doubt that large important structures exist in the lower crust and, more rarely, in the upper mantle, and it would be nice to think that they were in some way related to the surface geology and tectonics. At present this relationship is obscure. It is very difficult to extrapolate across the seismic blank zone that decouples almost every recognizable bit of surface geology from the structures in the lower crust and around the Moho.

A deep reflection profile across a young active continent-continent collision would be uniquely useful to students of the deep continental crust. To this end a small international group convened informally by Jack Oliver (Cornell University) and Matthews resolved that high scientific priority should be given to a profile across the Himalayan collision zone and Tibet. □

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Biochemistry

Evolutionary economics

from Roger H. Pain

ON PAGES 448 and 451 of this issue, two groups demonstrate homologies in amino-acid sequence in two different families of proteins. These similarities provide some clues about the evolutionary relationships of these proteins and both papers raise provocative questions concerning the economics of evolutionary processes.

A great many biomedical processes are powered by the energy derived from the hydrolysis of ATP. This requires a structure whose function is both to bind ATP and to enable energy transfer to the process concerned. The evolutionary development of such systems may be considered as having occurred in either of two ways, analogous to two different ways of powering an electronic circuit. In one, a 'power pack' can be designed on its own board to be plugged in to any circuit, needing minor modification only to accommodate different types of socket or connector. In the other, the components of the power supply are designed and built in as intrinsic parts of the circuit, needing no separate board or identity. Different arguments for economy in development can be advanced for each approach and it would be interesting to know which route has been followed in the evolution of biological structures involving ATP as their energy source. A further complication is that a component performing a single function in the power circuit could have been invented independently in different countries. The historian faced with the two versions has to decide whether the invention was independent or whether

one was developed from the design of the other. This latter problem arises in distinguishing between convergent and divergent evolution as the route by which proteins have acquired their present-day amino-acid sequences.

J. E. Walker and his colleagues (*EMBO J.* 1, 945; 1982) identified two consensus sequences, A and B, which although permissive, seem to occur in many proteins of diverse origin that are linked by the function of binding a mononucleotide, AMP or ATP (see figure). The sequences are

A K/R-space-G-(X)₄-G-K(T)

B K/R-(X)₃-G-(X)₃-L-(Np)₄-D

Consensus sequences associated with mononucleotide binding (from Walker *et al.*). The 'space' in A is largely non-polar. K, lysine; R, arginine; G, glycine; T, threonine; L, leucine; D, aspartic acid; Np, non-polar; X, any residue.

separated by about 100 amino-acid residues in this family of proteins, and no other sequence similarities have been identified.

Several different complex membrane-transport systems have a protein component in common which in each case shares sequence similarities as well as the Walker consensus sequence A. On page 448 of this issue, C. F. Higgins *et al.* show that this family of six transport-associated proteins also contains three other bacterial proteins associated with the very diverse functions of cell division, formation of surface nodules and export of the protein haemolysin. In addition to possessing the

rather highly conserved A sequence associated with ATP binding, the family described by Higgins *et al.* shares a surrounding 220–250-residue-long sequence containing other regions of high homology which distinguishes these proteins from the Walker family.

Higgins *et al.* are careful to point out that any evidence for the precise function of these nine proteins is sketchy but the present climate of opinion supports their suggestion that they all bind, if not hydrolyse, ATP. The overall homology suggests a further common function, possibly coupling the ATP-mediated energy to the cellular function of each enzyme complex. The ATP binding sites may owe their origin to the capture of a common ancestral gene fragment (which also encodes part of the site in the Walker family) and its insertion alongside a gene encoding another function. Alternatively, the precise stereochemical requirements of a mononucleotide site may have led to the independent selection of the appropriate amino acids within another gene which then diversified to match the different complex structures. The liberal variation in the consensus sequence makes the second alternative a real possibility.

Although it is fun to speculate in this way, it is unfortunately more difficult to justify scientifically while we have to rely solely on sequence information for clues to function as well as to evolution. Biochemistry must catch up with sequencing before we can argue from firmer ground.

On page 451 of this issue, R. F. Doolittle *et al.* take as their starting point the complex of protein units UvrA, UvrB and UvrC that aid the repair of damaged DNA by cutting the polynucleotide chain at two points separated by a dozen nucleotides. The nuclease is ATP-dependent and the UvrA component will hydrolyse ATP. Both UvrA and UvrB, but not UvrC, are known to contain the Walker consensus sequences for the binding of ATP. Doolittle *et al.* performed a computer analysis of the UvrA sequence that shows three pairs of sequences spread through its 940 amino acids, each pair containing the Walker A and B consensus sequences, respectively.

The first surprise is that this pattern of repeating putative ATP-binding sequence pairs has also been found in four other proteins, all of them part of the membrane-associated transport complexes considered by Higgins *et al.* This function seems a far cry from nuclease activity, but the pattern repeat is impressive and seems to argue for an ATP-binding power pack capable of modification to plug in to various biochemical effector systems. The fact that the transport proteins also belong to the Higgins family broadens the potential scope of this idea and nicely reinforces each paper.

Yet another intriguing fact emerges from the sequence scan of UvrA. Inter-

persed between the A and B consensus sequences is a second type of consensus sequence which seems to be associated with DNA binding and with metal binding. This sequence, CXXC-(X)_n-CXXC (where C is cysteine and X is any residue), occurs twice and, in addition, a vestigial trace of -CXXC- only occurs once. The two complete units occur in different environments relative to the ATP-binding A and B sequences. The ability to bind to DNA is of course an essential part of its function, so the A-sequence results are

gratifying. They will no doubt also lead to direct biochemical experiments that should help to clarify the mechanism of action of the excision nuclease. Doolittle *et al.* have already found that bound zinc is present in the Uvr proteins. The role of the different sites can now be probed, for example, by replacing key amino-acid residues using site-directed mutagenesis. □

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Neurobiology

Routes for development

from R. W. Guillery

A RECENT conference* that focused on the long-term changes in the nervous system demonstrated the extent to which some basic ideas may be applied to quite different levels of neuronal organization, from the cellular to the behavioural and from the earliest embryonic changes to the adult. It also showed that what we know about long-term changes is critically dependent on the techniques available, techniques that are currently being advanced, both by recombinant DNA methods and by improvements in imaging and staining methods; surgical procedures; and care in following definable neural relationships for weeks or months.

The broader relevance of the conference, which concerned the environmental influence on cerebral development, was addressed most particularly by a comparison of the development of cognitive and motor skills in normal infants with those

seen in apparently normal but prematurely born infants (F. Duffy, Harvard University). Even though the premature group was barely distinguishable from the normal, full-term group when tested initially at comparable post-conception ages, new evidence suggests that over a five-year period the pre-term group score lower in tests of complex behaviour patterns, although standard neurological examinations show no deficits. The extent to which premature, inappropriate sensory stimuli set the developing brain along abnormal developmental routes was briefly and tantalizingly raised.

The possibility that finer details of neuronal organization can be observed in a single experimental subject over extended periods of time was raised for single cells and synapses (D. Purves, Washington University, St Louis) and for complex cortical maps (M.M. Merzenich, University of California, San Francisco). Such

observations of single cells in adult rats have now been extended from earlier studies of the superior cervical ganglion, where long-term changes of dendritic arbors were shown, to surprisingly stable neuromuscular junctions, which show no continuous sprouting activity. Synaptic junctions in parasympathetic ganglia have only axosomatic junctions, so that axonal changes can in future be evaluated without confusion by dendritic changes. The vital staining methods being used for these studies are likely to be widely exploited.

In the cortical maps it appears that details can be altered by changes of the peripheral input. Peripheral nerve cuts or digital amputations can produce shifts of the evoked responses elicited from adjacent normal regions. These changes occur over distances of 500–1,200 µm but are thought not to be based on new anatomical connections. Either increased use or cross-innervations of two skin areas can also produce changes when evoked responses are studied by closely spaced microelectrode punctures at widely separated time intervals.

Studies of the neuromuscular junction and the climbing-fibre innervation of Purkinje cells illustrate general principles that may apply at all levels of development. At each site an early multiple innervation is reduced during normal development to a one-to-one relationship. In each situation this reduction is activity-dependent, the activity reduces the synthesis of a 'factor' for stabilizing synaptic relationships and competition between presynaptic fibres for a limited supply of the factor leads to the elimination of all but one of the fibres by a 'selectionist' process (J.P. Changeux, Institute Pasteur, Paris). Other speakers reported the role of chemical factors, of activity or of competitive interactions for the control of transmitter production in single sympathetic cells *in vitro* (E.J. Furshpan, Harvard; I.B. Black, Cornell); for the *in vivo* development of sexually dimorphic neural centres (A.P. Arnold, University of California, Los Angeles); and for the development of the visual pathways (see below).

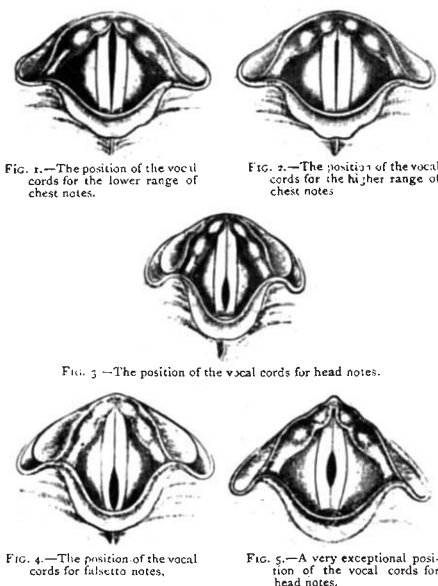
Sympathetic ganglion cells can be induced to synthesize one or more of four distinct transmitters (noradrenaline, acetylcholine, serotonin and substance P) in varying proportions; either specific chemical factors or membrane depolarization can influence the choice of transmitter production. The critical role of activity in neural development was demonstrated for the production of maps in the retinotectal pathways of fish (J.T. Schmidt, State University of New York at Albany) where both the normal sharpening of the map and the normal reduction of axonal arbor size are abolished by manipulations preventing normally correlated discharge patterns in the axons.

100 years ago

There is no question that the voice, whether the note be high or low, whether a chest or head note, whether bass or falsetto, is produced by vibration of the free edges of the vocal cords, which are two movable ligamentous bands about half and inch long stretched from back to front of the larynx. In other words, the only place where all notes, whatever their character may be, can be produced, is in the larynx.

The new and important observation which Dr. Mackenzie has made is, that in the head note of women and in falsetto singing only the anterior third of the vocal cords, as shown in Figs. 3 and 4, vibrate, and the remainder of the cords are in firm contact with one another.

The long-reed or chest voice is generally used by sopranos, Figs. 1 and 2 showing the position of the cords in the case of Mesdames Nilsson, Albani, and Valleria; on the other hand, the high notes of mezzo-sopranos and contraltos are short-reed, *e.g.* Madame Patey, as shown in Fig. 3. Tenors use both reeds, while the long only is used by the basses, and commonly by the barytones. Alto singers among men use the short-reed, whilst boys always use the long. In falsetto the false vocal cords, which are movable



able bands of tissue superior to the true vocal cords, also approximate considerably.

From *Nature* 34, 548; 7 October 1886

Classical experiments on visual deprivation and the formation of cortical ocular dominance columns (reviewed by T.N. Wiesel, Rockefeller University) were extended by the demonstration that here too patterns of impulse traffic are critical for the formation of segregated afferent territories (M.P. Stryker, University of California, San Francisco). I reported my own studies of Siamese cats which show that some genetically determined abnormal patterns of afferent activity can establish functional territories at subcortical but not at cortical levels, and the remarkable modifiability of the developmental programme of cortical and subcortical segments of these pathways was demonstrated by prenatal destructions of parts of the visual pathways of monkeys (P. Rakic, Yale University).

The possibility that an analysis of the development of complex motor patterns or perceptual capacities can usefully involve some of the same ground-rules that have been used for studying the development of single cells or of the intricate

retinofugal pathways was stressed by a consideration of the development of bird-song (P.R. Marler, Rockefeller University), which has a 'critical period' and involves the selection during maturation of a limited set of phrases from a larger initially acquired vocabulary; and by studies showing that one-month-old-human infants distinguish sound patterns used in speech as well as do adults, and on the basis of the same acoustic clues. During development the infant loses the capacity to make distinctions that play no part in its adult language (P.D. Eimas, Brown University). Although it is tempting to interpret developmental processes in terms of a few speculative generalizations such as selection, competition and activity dependence, a large gap still needs to be filled before cell biologists and behavioural biologists can share mechanisms as well as concepts. □

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Nuclear structure

Dizzily deformed dysprosium

from J.D. Garrett

THE recent observation of gamma-ray transitions between a sequence of superdeformed states in the rare-earth nucleus dysprosium-152 up to an angular momentum of 60 ± 2 units has caused considerable excitement among nuclear structure physicists. Not only does this measurement¹, made at Daresbury Laboratory, Warrington, United Kingdom, by a Daresbury/University of Liverpool/Niels Bohr Institute group, unambiguously establish superdeformed nuclear shapes,

but the technical achievement of finding a state of the nucleus with 60 units of angular momentum represents a 'quantum leap' forward in large angular momentum nuclear structure studies. The largest angular momentum previously reported² was 46 units, and recent advances have been in steps of one or two units^{2,3}. Indeed, 60 units approaches the angular momentum limit above which dysprosium-152 undergoes fission⁴.

About 2 per cent of the dysprosium-152 nuclei formed in the reaction of 205 MeV calcium-48 with a palladium-108 target populate the superdeformed states. For an angular momentum of 60 units the angular frequency of rotation is about 2×10^{20} revolutions per second. The nucleus slows down by emitting electric quadrupole gamma rays. The intrinsic structure of the nucleus, however, is not affected by this 'rotational braking'. A spectrum of the gamma-ray transitions between the sequence of superdeformed states depicting this process is shown in the figure.

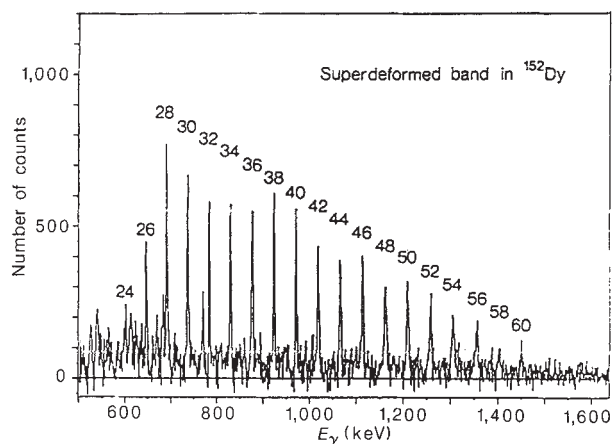
The constant separation of the gamma-ray energies

of neighbouring transition both confirms this picture and provides a measure of the nuclear moment of inertia. The observed 47-keV separation corresponds to a moment of inertia of a prolate ellipsoid with a major to minor axis ratio of 2:1 rotating about a minor axis. This is precisely the condition that produces a balance between the single-particle and collective nuclear effects that stabilizes the nuclear shape⁵. Indeed, for actinide nuclei, where the fission barrier is lower, delayed fission⁶ has been attributed^{7,8} to quasi-stable 2:1, or superdeformed, shapes.

The observations of superdeformed states allow the detailed study of three distinct coexisting shapes in dysprosium-152: slightly oblate ellipsoid⁹ (major: minor axis ($a:b$) $\approx 1.15:1$); prolate ellipsoid¹⁰ ($a:b \approx 1.25:1$); and superdeformed prolate ellipsoid ($a:b \approx 2:1$). An oblate ellipsoid has two major (longer) and one minor axes (the shape of a doorknob); the prolate ellipsoid has one major and two minor axes (egg-shaped).

The reported population of the superdeformed configuration in dysprosium-152 is greater than 100 times that of the most strongly populated 2:1 fissioning configuration in the actinides⁸. The enhanced population, coupled with improved arrays of high-resolution, low-background gamma-ray detectors becoming available^{11,12}, heralds the opportunity to study nature's only strongly interacting, many-body quantum system in a new extreme condition. Such studies have already been initiated at several laboratories in the United States and in Europe.

The myriad of questions concerning the details of the nuclear potential at very large deformations (for example, the radial dependence, the spin-orbit force, the angular-momentum dependence, higher-order terms and the existence of pair correlations, as well as the interaction of configurations corresponding to various coexisting shapes) led Nobel laureate Ben Mottelson recently to characterize nuclear structure physics as an immortal phoenix which is undergoing yet another reincarnation. □



Gamma-ray energy spectrum¹ showing discrete transitions between superdeformed states in dysprosium-152. The various gamma-ray lines are labelled by the angular momentum associated with the decaying level, which is uncertain within two units. (Courtesy of Daresbury Laboratory.)

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Molecular biology

Distinctions in acetylcholine receptor activity

from Edward Hulme and Nigel Birdsall

ON page 411 of this issue¹, Shosaku Numa and his colleagues bag another signalling macromolecule of neurobiological importance with the publication of the sequence and expression of a complementary DNA encoding a subtype of the muscarinic acetylcholine receptor from porcine brain. In 1914, Sir Henry Dale drew the classical distinction between the muscarinic and nicotinic actions of acetylcholine. The work of Numa's group now reveals the structural basis of this distinction.

Muscarinic acetylcholine receptors activate a set of heterotrimeric GTPases (G proteins) associated with the cytoplasmic surface of plasma membranes. G proteins mediate the effects of a diversity of receptors including photoreceptors, hormone receptors and neurotransmitter receptors. This suggests that such receptor molecules are variations on a common structural theme, an idea supported by the demonstration of sequence similarity between the β_2 -adrenoceptor and rhodopsin² and reinforced further by Numa's group¹, who now show that the muscarinic receptor also belongs to this family.

Activation of muscarinic receptors elicits a wide diversity of physiologically important biochemical responses including inhibition of adenylate cyclase, the phosphodiesteratic cleavage of polyphosphoinositides and the opening of a certain class of potassium channels. The α -subunits of the G proteins, G_i , G_o and G_{12} (?), that mediate these different responses must clearly show structural variation in their receptor and effector recognition domains³. There is evidence to suggest that there are differences in the sequences of the α -subunits in the carboxy-terminal region, which may be responsible for receptor recognition. Thus it may be possible to distinguish the functional subtypes of muscarinic receptors by their ability to interact selectively with the different G proteins.

The synthesis of conformationally restricted muscarinic ligands reveals complexities in the pharmacology and binding properties of muscarinic receptors. It is possible to distinguish at least three pharmacological subtypes of muscarinic receptors⁴, based primarily on their different affinities for the antiulcer drug pirenzepine and its cardioselective analogue AF-DX 116. In general it has not been possible to define a one-to-one relationship between pharmacological and functional subtypes, suggesting that two determinants may be at work.

When expressed on the surface of *Xenopus* oocytes, Numa and colleagues find that the muscarinic receptor DNA encodes a receptor which has a high affinity for pirenzepine. This might be taken to justify the conclusion that a M_1 muscarinic receptor subtype has been cloned and expressed. However, the affinity of selective muscarinic drugs is sensitive to the membrane environment of the receptor, so such a conclusion should be regarded as tentative. Nevertheless, the hypothesis that muscarinic receptor gene products with substantially different sequences are expressed in different cell types is supported by the presence of muscarinic receptor messenger RNA that will hybridize with this complementary DNA in the forebrain but not in the hindbrain and myocardium, although these are all regions where muscarinic receptors are expressed at moderate to high levels. What is not completely clear is whether these regional differences in hybridization result from the presence of receptors with different binding-site sequences, different effector (G protein) recognition sequences or to some completely unknown factor (for example, address sequence) associated with different cell types.

The amino-acid sequence of the muscarinic receptor complementary DNA has some features which were predictable from the functional analogy with rhodopsin. For instance, the sequence predicts seven transmembrane segments, often delimited by aromatic amino acids, whose general disposition is very similar to that found in rhodopsin and the β_2 -adrenoceptor. There is definite sequence similarity in these regions. There is a short amino-terminal sequence with two potential N-glycosylation sites which, in the mature protein, need to be extensively sialylated if the receptor is to be expressed on the cell surface.⁵ There is also a short, presumptively carboxy-terminal tail containing serine and threonine residues which may be target sites for cytoplasmic phosphorylation.

Transmembrane segments IV, V, VI and VII contain proline residues which are conserved in all three proteins, as they are in some of the short connecting loops, suggesting close similarities in the three-dimensional structures. There are several cysteine residues which could participate in interhelix bonding. Of interest is the evidence of internal sequence duplication between transmembrane segments I, II and III but not between IV, V, VI or VII.

The most striking feature of the muscarinic receptor sequence is the presence of a long (approximately 100 amino-acid) insert in the cytoplasmic loop joining transmembrane segments V and VI. The amino-terminal half of this insert contains runs of serines and acidic residues and is enriched in proline and glycine residues. Similar sequences have been noted in the sodium channel, in viral transforming proteins and in homeotic gene products, but their function is unknown. Interestingly, the carboxy-terminal portion of this loop is enriched in basic amino acids, which are also abundant in cytoplasmic loop III-IV, and in the tail. In fact, basic residues are much more abundant in the muscarinic receptor sequence than in the β_2 -adrenoceptor or rhodopsin. Indeed if it were not for the negatively charged sialic acid residues, the protein would bear a net positive charge.

The asymmetric charge distribution makes the molecule highly dipolar, probably with a strong tendency to orient in the electric field across the membrane and a minimal tendency to dimerize or cluster on the cell surface. However, phosphorylation of serines and threonines on the cytoplasmic loops and tail could modify this property. In the case of the β_2 -adrenoceptor and opsin, such phosphorylation is involved in the down-regulation of the receptor. Another interesting point is that the lysine and arginine content of the purified porcine myocardial receptor⁶ seems to be approximately 50 per cent of that of the cortical muscarinic receptor, reinforcing the idea that the cortical and myocardial receptors may be different gene products.

The ability of pharmacologically distinct receptors to activate the same G proteins suggests that they may be composed of a ligand binding domain linked to a transduction domain. In the case of opsin, proteolysis studies suggest that the tail region and the cytoplasmic loops joining transmembrane segments III-IV and V-VI may be involved in G-protein recognition⁷. Superficially at least, however, the greatest sequence differences occur in these regions. Despite these differences, both opsin and, as shown by Haga, Haga and Ichiyama⁸, the porcine muscarinic receptor can efficiently activate the adenylate cyclase inhibitory G protein G_i . Does this imply a role for the more highly conserved transmembrane

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regions in G-protein recognition?

Another crucial question is the location of the ligand binding site. In opsin, the retinal chromophore forms a Schiff's base with a lysine, buried in transmembrane segment VII. However, in the case of the muscarinic receptor, and indeed several other monoamine receptors, there is evidence that carboxylate groups are involved in binding the positively charged ligands. Our group has found that labelling the binding site of a purified rat brain muscarinic receptor with the irreversible antagonist propylbenzilylcholine mustard, followed by proteolytic digestion, yields two peptides whose properties sug-

gest that they are labelled on acidic residues in hydrophobic regions⁹. One of the peptides is glycosylated and so is likely to come from the amino-terminal half of the molecule. It is intriguing to find two buried aspartate residues, one in transmembrane segment II and one in transmembrane segment III, which are conserved in the β_2 -adrenergic receptor sequence. Are these key residues in the muscarinic ligand binding site? □

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Mathematics

Singular flying pancakes

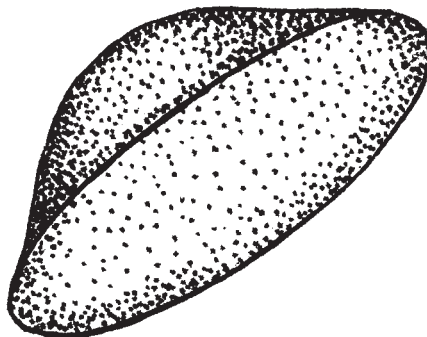
from Ian Stewart

IF YOU look into a cup of coffee on a summer's day you can often see a bright curve with a sharp tip, where the light is focused after reflecting off the shiny surface of the cup. Such a curve is called a caustic, because heat, as well as light, is concentrated there.

Effects analogous to caustics do not occur solely in optics. The sonic boom of a jet aircraft, and even louder 'superboom' when it turns a corner, are acoustic caustics. So are the signals received by geologists when seeking oil-bearing strata by remote sensing. There are even caustics in quantum mechanics and, perhaps, in the formation of galaxies. J.B. Zeldovitch's model of galaxy formation (*Russian Math. Surv.*, *Uspekhi* **30**, 204; 1975) is based on matter condensing along shock waves. The Zeldovitch theory appears as an example in a celebrated paper of V.I. Arnold (*Commun. Pure Appl. Math.* **29**, 557; 1976) on the singularities of an evolving wavefront. This is the general mathematical setting in which to pose questions about caustics in all of their manifestations. Arnold assumed that the velocity field is governed by a potential, which allowed him to apply general results of V.M. Zakalyukin (*Itogi Nauki* **22**, 53; 1983). Now J.W. Bruce has analysed the non-potential case (*J. London Math. Soc.* **33**(2), 375; 1986) an extension made possible by recent results in singularity theory due to Bruce, A.A. du Plessis and C.T.C. Wall. The main effect of the generalization is to exclude some possible forms for the condensing surfaces that arise in the potential case, and to emphasize instead two new ways in which these surfaces can change their form.

The basic model is as follows. Consider a continuum of non-interacting particles in space, with a smooth initial velocity distribution and a smooth density distribution. As time passes, the particles will

move inertially along straight-line paths. These paths can intersect at so-called singularities. By analogy, think of the paths as light rays: the singularities are the caustics, where rays come to a focus. Thus 'collisions' of particles will produce a singular surface in this flow, analogous to a shockwave; and matter will tend to condense around this surface. The mathematical problem, which applies to various



The flying pancake is the simplest way that a singularity can evolve in a continuum of non-interacting particles. It may describe the initial condensations of matter in the Universe.

physical situations, is to classify the types of singularity that can occur.

In the potential case the initial velocity field is the gradient of a smooth function, and the singularity is mathematically equivalent to an optical caustic. The simplest singularity that can occur is the 'flying pancake', a lens-shaped surface with a cusped edge and a dent on one side. Initially there are no condensation surfaces; but, then a tiny pancake appears, which grows rapidly (see figure). In a cosmological setting this was interpreted as the formation of a lenticular galaxy, but it is now felt that it more arguably represents the formation of a cluster of galaxies.

How justifiable is the assumption of non-interaction? According to Arnold, in

his delightful and unusually readable *Catastrophe Theory* (Springer, Berlin, 1984), it is certainly reasonable up to the point at which pancakes first form. After that it depends on the proportion of neutrons in the Universe, and is justified provided they account for a significant part of the matter present.

The new results of Bruce show that in the more general non-potential case the simplest singularity is still the flying pancake. He obtains a list of nine possible 'typical' ways for the singularity of the wavefront to evolve, seven of which also occur in Arnold's list for the potential case. Three singularities on Arnold's list become atypical in non-potential systems and in practice will break up, in a way described by the remaining two singularities on Bruce's list. Except at this transition, there is no significant difference between the potential and the non-potential cases. The extent to which these ideas really apply to the formation of galaxies is not yet clear, but Zeldovitch's theory does help to make sense of recent observations that matter in the Universe tends to cluster on surfaces, surrounding huge voids. In this picture the pancake (see figure) represents a cluster of galaxies. But the mathematical setting is very natural, and the theory should provide a broad guide to the general types of morphology expected, and a suitable framework for organizing more detailed analyses.

The relation of the mathematical methods to their applications is interesting. Singularity theory is a subject that has grown explosively over the last decade. To cut a long story short, it may be viewed as the mathematical legacy of catastrophe theory, proposed by René Thom in the late 1960s as a model of biological (and other) morphogenesis, and developed by Thom and many other mathematicians (notably J. Mather, B. Malgrange and Arnold and his collaborators). The subject has led a double life, in part as a branch of pure mathematics marching to its own drummer, and in part as a programme guided by various physical and biological problems. An analogy between 'morphogenetic fields' in biology and optical caustics provided some early motivation. Moreover in some sense it has always been clear — or should have been — that singularities are likely to be important in applied science. However, the main techniques used, without which nothing effective can be done, are of purely mathematical origin and were not devised with any specific physical purpose in mind. Anyone who believes that the way to solve a problem is to mount a frontal attack should take a close look at the convoluted but creative history of this particular interaction between mathematics and science. □

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Ecology

Predicting fruitfly guild sizes

from John H. Lawton

ONE aim of theoretical ecology is to predict the number of species able jointly to exploit a common resource in a similar fashion, that is to predict guild sizes¹. Traditionally, the problem has been approached by partitioning the requirements of each species within the guild along some 'resource-axis'. By determining the total supply of resources, and each species' requirements and overlapping demands, the number of species able to coexist can be predicted^{2,3}, at least in theory. This yields some interesting insights, but the difficulties of estimating the intensity of competition between species makes quantitative predictions about real guilds appear hopelessly difficult, not least because of the snowstorm of parameters required (for example, ref. 4). But new work^{5,6} appears to be much more successful in predicting the number of species that can coexist within a guild.

One alternative approach retains the emphasis on interspecific competition, expressed by the competition coefficients of the Lotka–Volterra equations^{2,3}, but avoids the complexities of the field by use of controlled experiments in the laboratory. Fruitflies (*Drosophila*) are suitable animals to work with because large numbers of different species can be kept in pure cultures and guilds of various sizes can be assembled by pooling populations of any number of species. This approach is the one adopted by Michael Gilpin and his co-workers in California⁶ over the past 10 years. The results are interesting in many

ways, but in the present context are actually rather simple. Starting with mixtures of 10 different species, over a range of initial densities, the cultures always collapsed to a maximum of no more than three species (7 out of 30 trials), and usually less (two coexisting species in 21 trials, and a single victor in one other).

Intriguingly, real *Drosophila* guilds in the field are conspicuously larger than this. The 'field' in this case consists of a variety of small, patchily distributed, ephemeral breeding sites used by *Drosophila*, ranging from rotting fruits and sap flows to fungal fruiting bodies and various flowers. In samples of 53 such sites from tropical and temperate habitats all over the world, Bryan Shorrocks and Jonathan Rosewell⁶ find a modal guild-size of seven species. The reason why real *Drosophila* guilds are generally at least two or three times the size of laboratory guilds carries an important message for community ecology.

Gilpin's laboratory experiments deliberately exclude spatial heterogeneity because its inclusion would seem likely to make both experiments and models more difficult to construct and interpret. But paradoxically, as Shorrocks and Rosewell show⁶, incorporating spatial heterogeneity actually makes it easier and simpler to predict guild sizes, at least for *Drosophila*. Their approach also shifts emphasis away from competition coefficients as the key to understanding guild sizes, to the spatial distribution of species across resources.

For community ecology this is a dramatic change of emphasis. As Shorrocks and his colleagues show⁷, the spatial distribution of individual *Drosophila* species across breeding sites is highly clumped, a pattern that can be described simply, albeit somewhat crudely, by the negative binomial distribution.

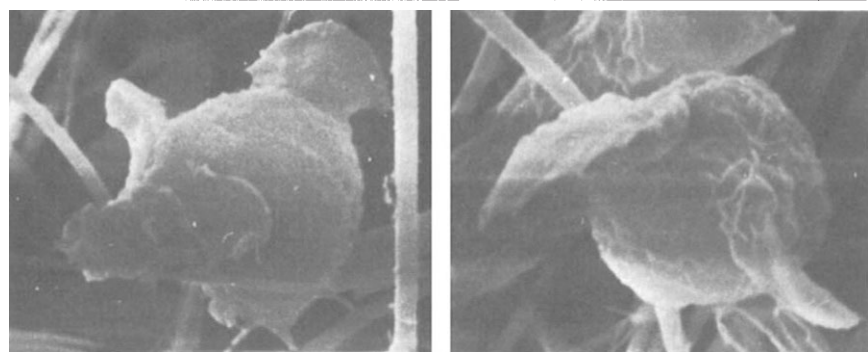
As long as different species of *Drosophila* have different, independent but sufficiently aggregated distributions, it is possible for more than two or three species to coexist quite happily in one type of breeding site. Even more interesting, a model of the interactions between several species in a spatially patchy world, incorporating sensible parameter values derived from real *Drosophila* populations, predicts a modal guild size of five or six species, encouragingly close to the observed number of seven. The frequency distributions of observed and predicted guild sizes are also reasonably similar, ranging from two species to about twenty.

The model is simple and works by allowing species to 'invade' the community sequentially, in order of their competitive dominance. Breeding sites left unused because of the clumped distribution(s) of the superior competitor(s), are then invaded by inferior species, until further invasion is no longer possible. However, the spatial refuges so created are not analogous to resource partitioning in more traditional models of community ecology, because each species redistributes itself after each generation, and distributions are not related to particular habitat variables or resource-axes such as moisture or degree of rotting. Nor is the outcome crucially dependent on the magnitude of the competition coefficients; more important is the degree to which species aggregate in their use of breeding sites.

This is very different from the previous ways in which ecologists have tried to understand how guild sizes are determined, and much more successful. It remains to be seen whether this kind of model will be useful for predicting guild sizes in other types of animals, and whether the match between observed and predicted guild sizes in *Drosophila* is roughly right for completely the wrong reasons. My own guess is that it is too good to be coincidence. □

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THE lineage fidelity of interleukin-3-dependent cells has for some time been the cause of uncertainty. In a recent private discussion, these cells were described as 'pigs to work with' and elsewhere I heard the view expressed that varied behaviours of these cells are 'somewhat horny'. While investigating the morphology of certain interleukin-3-dependent cell lines (left: AC2, 4,050 $\times$; right: 123, 2,550 $\times$), I can confirm that porcine and ovine attributes are indeed displayed by such cultures, as shown in the figure. In addition, there is a small but significant representation of whales, dolphins or tuna within the populations (data not shown), from which I conclude that interleukin-3-dependent cells may be somewhat fishy, and may perhaps need a pinch of salt in certain circumstances.

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Core fragments in Chernobyl fallout

SIR—Among the first reports¹⁻³ of the Chernobyl fallout were references to the presence of localized areas of high radioactivity, called hot spots, largely on the basis of measurements of γ radioactivity in fallout outside the Soviet Union. We now have evidence for α radioactivity from hot spots inadvertently collected in the Soviet Union by travellers from abroad. The γ -ray and α -particle spectra are those expected from fragments of fuel rods. We also have indications that such particles may have spread into the north-eastern part of Poland.

Having learned of our group's work, Dutch travellers returning from the Soviet Union and other East European countries gave us the opportunity to inspect their clothes and other belongings. In some cases, we also made detailed measurements of luggage. We observed enhanced radioactivity in the following: a pair of trousers from a person who spent some days in Kiev at the time of the accident towards the end of April, a pair of trousers of a boy who had camped in north-east Poland in July and a shoe from a girl who had visited Minsk at the beginning of August.

On the trousers from Kiev, we found at least five areas with intense localized γ -ray activity, the shoe from Minsk contained one such area and the boy's trousers contained various spots with enhanced

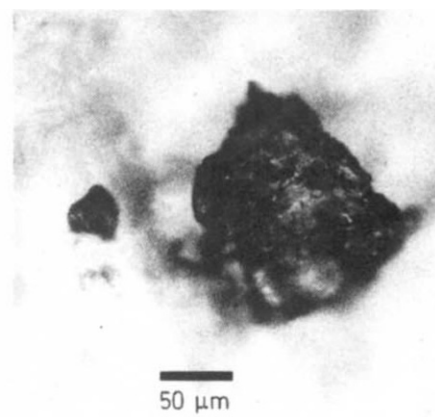
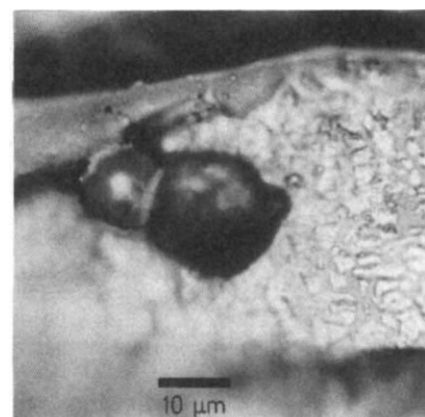


Fig. 1 Micrographs of what is probably a hot particle from the Kiev trousers (left) and the Minsk shoe (right).

radioactivity. All the spots were localized with a sensitive hand monitor for gamma and beta radiation and the gamma-ray spectra were measured with a Ge detector (Table 1). It will be seen that the relative abundance of the γ -ray emitting nuclei for all three subjects is practically the same and that the activity ratio of $^{134}\text{Ca}/^{137}\text{Ca}$ of about 0.5 is that characteristic of the Chernobyl fallout^{2,3}. But the proportions of ^{95}Zr and ^{144}Ca are much higher than in the fallout reaching Western Europe³, suggesting that the particles we have measured were released in the first plume, which reached Scandinavia.

With pieces of adhesive tape, we were able to remove one hot particle from the Kiev trousers and another from the Minsk

shoe. Both particles were studied under a microscope and photographed (Fig. 1). More detailed photographs show areas of crystalline as well as amorphous material, indicating that some melting must have taken place. The activity on the trousers from north-east Poland, most probably contained in finer particles, was too low to isolate.

The sizes of the particles from Kiev and Minsk were determined to be approximately $30 \times 15 \times 3 \mu\text{m}$ and $150 \times 150 \mu\text{m}$ respectively. The α spectra from these particles are almost identical: a continuous increase from 2.5 MeV to 6 MeV, where the spectrum has a sharp high energy edge. The total α activities were 5 and 1.4 Bq from the Kiev and Minsk particles respectively.

To obtain high-resolution α -spectra, the particle from Minsk was dissolved in hydrochloric acid and the radionuclides precipitated onto a stainless steel disk by electroplating (Fig. 2). The dominant activity at 6.12 and 6.07 MeV is consistent with ^{242}Cm , while the smaller peaks at 5.16, 5.50 and 5.79 MeV are consistent with the presence of $^{239,240}\text{Pu}$, $^{243,244}\text{Cm}$, ^{238}Pu and, possibly, ^{241}Am . But the shape of the peak at 5.50 MeV indicates that ^{238}Pu (decay product of ^{242}Cm) is the dominant contributor. It is clear (Table 2) that the activities correspond to only a fraction of the total mass of the original particles.

From the concordance of our measured α and γ emitting nuclides with the estimated inventory of a reactor core⁴⁻⁶, the characteristic Chernobyl radio $^{134}\text{Ca}/^{137}\text{Ca}$ and the similarity of the total α and γ spectra of the Minsk and Kiev particles, we conclude that both hot particles are fragments of the core of the Chernobyl reactor released during the accident. Thus the temperature must have been high enough partly to melt the fuel rods, which is in accordance with earlier reports from Sweden¹. The fragments have contaminated a large area from at least Kiev (150 km leewards) to Minsk (400 km downwind of Chernobyl).

Table 1 Fractional γ -ray activities (%) for various radionuclides in the hot particles of the "Minsk shoe" and the "Kiev trousers", and the "complete" trousers of NE Poland

Nuclide	Kiev trousers*	Minsk shoe†	NE Poland trousers‡
^{141}Ce	25	29	30
^{144}Ce	22	24	17
^{103}Ru	24	21	25
^{134}Cs	1.0	1.1	0.3
^{137}Cs	2.4	1.6	0.7
^{95}Zr	26	24	27
Total α -ray activity§ (Bq)	850	420	41

Activities are corrected for decay since 26 April.

* Measured on 7 July 1986.

† Measured on 13 August 1986.

‡ Measured on August 24 1986 for the complete trousers.

§ Total γ -activity determined only from the nuclides listed.

Table 2 α Activity of the hot particle from the Minsk shoe

Nuclide	$t_{1/2}$ (yr)	E_{α} (MeV)	α -Activity (Bq)	Mass (μg)
$^{238}\text{Pu} +$ ^{240}Pu	24,400 6,580	5.12–5.17	0.070	$< 2.9 \times 10^{-5}$
$^{238}\text{Pu} +$ ^{241}Am	86 458	5.45–5.50	0.080	$< 6.2 \times 10^{-7}$
^{243}Cm ^{244}Cm	32 17.6	5.74–5.81	0.021	$< 1.2 \times 10^{-8}$
^{242}Cm	0.447	6.07–6.12	2.0	1.6×10^{-8}

The α activity was determined on 29 August, and has been corrected for decay since 26 April.

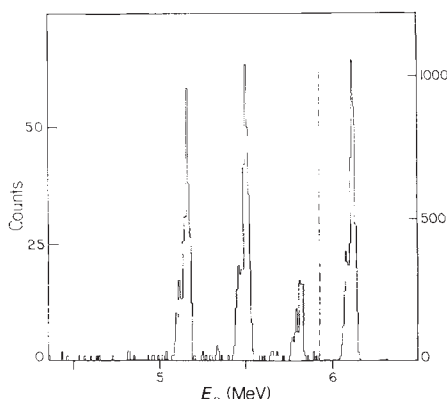


Fig. 2 Energy spectrum of α particles emitted by the hot particle on the Minsk shoe. The spectrum has been taken after dissolving the particle in HCl and precipitating the radionuclides on a stainless steel disk by electroplating.

While our measurements say nothing quantitative about the degree of contamination of the area or the percentage of the core that has been emitted, the presence of at least five hot particles on the trousers from Kiev and the fact that at least one hot particle was collected during a twelve-hour stay in Minsk in August indicate that the situation warrants further investigations.

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Microtubule assembly in the axon

SIR—Solomon's interpretation of our data² is possible but, as we have stated, it requires microtubule assembly at the cell body to be resistant to the application of antimicrotubule agents to an unprecedented degree. It was pointed out many years ago that phenomena involving ongoing microtubule polymerization (such as mitosis and axonal elongation) are extremely sensitive to colchicine and related drugs³. Therefore a region that displays a more than 100-fold greater sensitivity to such drugs over other parts of the cell must be considered to be a prime candidate for the assembly process.

Evidence in favour of the widely accepted alternative model of assembly at

the cell body is also indirect. Essentially, it depends on the observation that newly synthesized tubulin molecules rapidly enter a form in which they are non-diffusible and not extracted by non-ionic detergents (for example, refs 4, 5). The nature of this form has not, however, been characterized further.

More experiments are certainly needed. We hope that our observations, together with Dr Solomon's cogent comments, will lead to an early resolution of this intriguing question.

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Biochemical basis of cystic fibrosis

SIR—Readers of Maynard Case's *News and Views* article¹ on cystic fibrosis (CF) may be interested in our *in vitro* studies of protein secretion from submandibular tissues in CF.

We have reported^{2,4} a markedly decreased stimulation of protein secretion in two CF submandibular glands by the β -adrenergic agonist isoprenaline, and have since confirmed this observation with a third sample⁵. We have also shown^{3,4} that in the CF tissues isoprenaline increases cyclic AMP levels to the same extent as in control tissues and that β -adrenergic responsiveness is partially restored by the cyclic nucleotide phosphodiesterase inhibitor, 3-isobutyl-1-methyl-xanthine.

On the basis of these results, we proposed that the CF defect was at an intracellular regulatory site, distal to the generation of cAMP, possibly in cAMP phosphodiesterase activity. In view of observations of defects in calcium homeostasis in various cell types from CF patients⁵ and that cAMP phosphodiesterase represents a point of interaction of the cAMP and Ca^{2+} regulatory pathways, we further proposed that the defect may reside in altered function of a Ca^{2+} -dependent regulatory protein, such as calmodulin. To test this hypothesis, we have isolated calmodulin from control and CF submandibular glands and found that control and CF calmodulins run with identical elution time on reversed-phase high pressure liquid chromatography, suggesting that there is no marked structural alteration in CF calmodulin.

In addition calmodulin activation of cAMP phosphodiesterase by crude ex-

tracts from CF cells is increased relative to control extracts, suggesting an alteration in a specific modulator of calmodulin function in CF cells. This could affect cAMP-mediated responses which interact with Ca^{2+} but not necessarily other Ca^{2+} -mediated events such as Ca^{2+} -activated K^{+} channels in airway epithelia, which have been reported to be normal in CF⁷.

The finding of defective β -adrenergic stimulation of Cl^{-} transport^{6,7} and protein secretion^{2,5} in affected CF tissues suggests that alteration in a common intracellular mediator or inhibitor protein is more likely to cause this disturbance than an abnormal site on the Cl^{-} channel itself.

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Silver foils gravitational principles

SIR—Walter Gratzer, in his review of Irving Klotz's book (*Nature* **322**, 781; 1986) quotes some examples of credulity and ignorance among which Klotz writes, it seems, that "From experience with non-science college students, I know that the majority predict that if a silver dollar and a silver dime are simultaneously dropped from the top of the Sears Tower the former will hit the ground first".

But if a silver dollar and a silver dime are dropped from the top of the Sears Tower (or from the top of the Leaning Tower of Pisa) the silver dollar will hit the ground first, the silver dime will get down second and a disk of silver foil will flutter to the ground a poor third.

College students, whether of science or non-science, may sometimes be right without knowing why and their teachers may sometimes be wrong.

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What's going on in the cell?

Joseph G. Gall

Molecular Cell Biology. By James Darnell, Harvey Lodish and David Baltimore. W.H. Freeman: 1986. Pp. 1,187. Hbk \$42.95, £42.95; pbk £19.95.

ALTHOUGH many of the principles of cell biology were well established a generation or more ago, the sheer volume of molecular data gathered in recent years has transformed the subject. As usual in a rapidly advancing science, the explosion of information has resulted from an interplay between new analytical tools and new ways of thinking. The new tools include sophisticated methods of studying minute amounts of macromolecules — ultracentrifugation, new microscopes, X-ray diffraction, protein and DNA sequencing — as well as radically new ways of using biological materials, such as monoclonal antibodies, viruses, tissue cultures and gene clones. The new framework of thinking comes from an understanding of the basic architecture of biological macromolecules, particularly the nucleic acids and proteins, and their prime role in all cellular processes. The number of these processes about which we now have detailed molecular information is legion — cell motility, transport of molecules across membranes, ligand-receptor interactions, antibody formation, chromosome organization, RNA transcription and processing, protein synthesis, electrical properties of membranes, and energy conversion, to name only the more important ones.

Molecular Cell Biology takes the whole gamut of cell structure and function as its subject, and makes forays into developmental biology and evolution at the end. The result is a large book, packed with detailed, up-to-date information and illustrated with numerous excellent diagrams and photographs.

There are 25 chapters, grouped under four major headings ("Introduction: Molecules, Cells, and Experimental Techniques"; "Gene Expression, Structure and Replication"; "Cell Structure and Function"; and "Normal and Abnormal Variations in Cells"). To a certain extent the first three headings include subjects that are usually taught in beginning biochemistry, molecular genetics and cell biology courses; but the authors have successfully blended topics together in order to emphasize how biochemistry and genetics relate to workings at the cell level, both prokaryotic and eukaryotic.

In an enterprise such as this there must

be compromise between the demands of comprehensiveness, depth, historical perspective and up-to-the-minute data. In general the authors have opted for as much recent information on as many topics as possible, without getting into the complex questions of how we got here or who did what, when. This is an understandable and reasonable approach, but cell biology, even molecular cell biology, has an interesting intellectual history that is an important ingredient in understand-



Pointing the way — Francis Crick and James Watson examining the double-helical model of DNA built in 1952–1953.

ing the current state of affairs. Some topics are covered with this sense of history when they are the special province of one of the authors. For instance the discussion of RNA processing leads one carefully through the logic and development of the field, stopping to consider false starts and problems along the way. It is important for students to learn from such examples how science really proceeds. I would have preferred more of this type of presentation, with some acknowledgment of the people involved.

Within each chapter there are numerous headings (in black type) and subheadings (in red type), to help students identify major topics and manage an otherwise overwhelming volume of information. The red subheadings are in the form of declarative statements, a style increasingly popular in textbooks and in titles of journal articles. Such statements pose two dangers: first, they may overstate what is known; second, their grammatical similar-

ity may obscure differences in logical structure. For instance, the following are all red subheadings in *Molecular Cell Biology*: "Catecholamines are widespread neurotransmitters." "The nuclei of differentiated frog cells can retain complete developmental potential." "The endosymbiotic hypothesis is confirmed by rRNA analysis." These are in no sense equivalent statements; training in any science, not just cell biology, should teach one to distinguish between them and to evaluate the different kinds of evidence and degrees of certainty involved.

The authors say their book is written for use in a one-year course that integrates molecular biology with biochemistry, cell biology and genetics, and applies this coherent insight to the problems of development, immunology and cancer. There is probably too much material in this book for a single undergraduate course. Nevertheless, the level is appropriate, and instructors can pick and choose among the chapters. Advanced students and practising cell biologists will all want to have a copy for reference and for catching up in areas outside their own expertise.

It would be impossible to write this review without comparing *Molecular Cell Biology* with *Molecular Biology of the Cell*, published three years ago by Alberts, Bray, Lewis, Raff, Roberts and Watson. Besides the similar titles, the two books have the same dimensions, are of equal length and each has a shiny black cover featuring a single tissue-culture cell glowing by immunofluorescence (not to mention that each has a Nobel laureate among the authors). The similarity extends even further, including the extensive use of diagrams, the style of writing, the range of topics and the intended audience. There are differences — the Alberts book has a bit more on development and a special chapter on plant cells, while Darnell and his colleagues devote chapters to cancer and the evolution of cells. But the similarities far outweigh the differences. Three years ago in a review of *Molecular Biology of the Cell* (*Nature* 302, 637, 1983) I stated that it would be a long time before you could find a better buy. Now I can say that you have the choice between two excellent, comprehensive and up-to-date texts, and you will have to make up your own mind between them. Better still, get them both. □

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Groundwork on the troposphere

Richard P. Wayne

Atmospheric Chemistry: Fundamentals and Experimental Techniques. By Barbara J. Finlayson-Pitts and James N. Pitts, Jr. Wiley: 1986. Pp. 1,098. £57.45, \$59.95.

TWENTY-five years ago Philip Leighton's seminal monograph *The Photochemistry of Air Pollution* was published. Leighton applied basic chemical kinetic, photochemical and spectroscopic concepts to problems of tropospheric air pollution. A striking development of the 1950s had been the recognition that photochemical reactions initiated by sunlight could convert relatively innocuous primary pollutants into substances that created health hazards, nuisance and economic loss. One of Leighton's outstanding contributions was in bringing together the available knowledge about chemical processes and absorption rates in the atmosphere in an attempt to describe the conversion of the initial pollutants to final products, the underlying motivation being not only the scientific interest of the problem but also the possible applications to the control of pollution.

Since 1961, an enormous amount of research has been conducted in the laboratory on chemical transformations, and in the field on sources and concentrations of pollutants. Computer models have made possible realistic chemical bases for pollution control strategies. Jim Pitts and Barbara Finlayson-Pitts have been major contributors to much of this research. Their new book is a worthy successor to Leighton's, adopting a similar approach that is strongly grounded in laboratory chemical experiments.

The atmospheric chemistry treated is discussed mainly with the Earth's troposphere, although the impact of tropospheric chemical processes on stratospheric ozone is considered briefly. The thrust of the book is clearly directed towards the sources, chemistry and control of air pollution. The particular topics discussed include photochemical smog, acid deposition, toxic volatile organic compounds, airborne mutagenic polycyclic aromatic hydrocarbons, and primary and secondary particles in the atmosphere. However, the authors show that there is a close interrelation between these various manifestations of pollution, the photochemical and thermal reactions of a relatively small number of species making up the overall complex chemistry and physics. In many cases, the "polluted" troposphere shows in exaggerated form the chemistry of the natural troposphere. For

example, the ozone and the oxides of nitrogen that are features of photochemical smog are important species in the troposphere remote from man's activities and air pollutant emissions, and sulphur compounds and hydrocarbons are released to the atmosphere by natural biogenic processes. The authors therefore review the chemistry of the natural troposphere as a special topic.

Roughly half of the book is devoted to presenting the principles of kinetics, spectroscopy and photochemistry that are applicable to atmospheric chemistry, and to the kinetics and mechanisms of important specific gas phase reactions in real and simulated atmospheres. Other important parts of the book deal with modelling studies, and with the physics and chemistry of aerosols and the mechanisms of their formation. I particularly appreciated the descriptions throughout the book of how experiments are really performed. For example, there is a comprehensive discussion of flow, flash, modulation and static

techniques for determining rate constants in the gas phase, in solution and on solid surfaces, together with descriptions of methods for the generation and detection of reactive reaction intermediates. Similarly valuable accounts are given of techniques for monitoring pollutants, the use of environmental ("smog") chambers and the analysis of particles.

By any standards this book is a major work. It is authoritative and comprehensive. The text abounds with tables and figures of factual data to back up the themes presented, and the references provide an overview of research published up to mid-1985. *Atmospheric Chemistry* will long remain a valuable text and reference book describing the chemistry of the gases, droplets and particles present in the natural and polluted troposphere. □

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Myths of Mars

James Trefil

To Utopia And Back: The Search for Life in the Solar System. By Norman H. Horowitz. W.H. Freeman: 1986. Pp.168. Hbk \$17.95, £17.95; pbk \$11.95, £11.95.

"THE idea of an inhabited Mars occupies a special place in our culture." With this comment, Norman Horowitz touches off a short but fascinating discussion of the process by which our modern view of Mars was forged. As the former head of the bioscience section of the Mariner and Viking missions, he had a ringside seat during that exciting period in the 1970s when the human race, for the first time, sent probes to the surface of our sister planet.

This is not, however, a dry recitation of technical achievements, although the setting up of a chemistry laboratory capable of operating and sending back useful information over interplanetary distances is surely one of the great technical feats of all time. Instead, Horowitz takes us on a tour of the development of our ideas about the origin of life, from spontaneous generation to chemical evolution, showing at each step how misconceptions about life tricked people into believing that all the planets of the Solar System were populated by living beings. He deals with and sets the record straight about things such as the canals of Mars and Percival Lowell's widely read views on Mars as an abode of life. He then carries this discussion forward into the 1960s, when a prestigious panel assembled by NASA stated that the Martian atmosphere was similar to our

own. It seems that whenever we have had a chance, we have interpreted our data in such a way as to produce a picture of an Earth-like Mars.

In the modern debate over the existence of extraterrestrial intelligence, this proclivity has been elevated to something called the "Principle of Mediocrity", which states that the Earth does not occupy a special place in the cosmos. This principle is accepted without proof — indeed, for some writers it has taken on the mantle of dogma. Yet, as Horowitz shows, when it is applied to the one body of which we have extensive knowledge, it fails miserably. In a masterly summary of the search for life on Mars by the Viking landers, he discusses each of the experiments carried out and shows how they establish that Mars, with its highly reducing environment, is far more hostile to life than we had ever imagined.

Of course, a few diehards refuse to give up the ancient myth of an inhabited Mars. Horowitz dismisses their theories, rightly I think, as "daydreams", and variations of the "blue unicorn theory".

All in all, this is a well-written, non-technical book. Anyone who, like myself, has followed Martian exploration at a distance, and, would like to have a clear, concise summary of what we now know, will find it makes excellent reading. I only wish that Horowitz had told us a little more about his own emotions and reactions as this crucial step in mankind's knowledge of the Solar System was made. □

James Trefil is a Professor in the Department of Physics, University of Virginia, Charlottesville, Virginia 22901, USA. His latest book is *Meditations at 10,000 Feet* (Charles Scribner's Sons, 1986).

Alimentary ideas

Stuart Sutherland

The Psychology of Eating and Drinking.
By A.W. Logue. W.H. Freeman:1986.
Pp.298. Hbk £24.95, \$24.95; pbk £14.95,
\$14.95.

EATING and drinking may seem quite straightforward activities, but they are associated with a vast range of phenomena: from bulimia (binge eating) to anorexia nervosa; from the development of a palate for fine wines to pica (the repeated consumption of non-nutritive food like clay or chalk); and from the increase in food consumption when it is followed by pleasurable circumstances to the remarkable capacity of animals and man to develop an aversion to any food whose consumption is followed — even several hours later — by sickness. In *The Psychology of Eating and Drinking*, Professor Logue reviews all these and many other equally fascinating phenomena.

Despite the vast amount of work on eating and drinking undertaken over the past three decades, we have little secure understanding of these activities. It is, for example, over 30 years since J. Mayer proposed his theory of hunger. He suggested that in the short term it is controlled by the rate at which glucose is being removed from the blood, as measured by the difference in its concentration in arteries and veins. Such a mechanism could not maintain a constant body weight, so he added a long-term control system which, through the measurement of fat metabolites, turns hunger on when fat is being consumed and off when it is not, thus keeping the amount of body fat stable.

These theories were largely disregarded for many years, and have only recently received the attention they deserve. There is now evidence to support them, although the physiological details have not been unravelled and nothing is known about how the short- and long-term systems interact. Moreover, the control mechanisms are much more subtle than is hinted at in Mayer's theory. They include anticipatory eating and drinking, that is eating and drinking before there is any physiological deficit if there is an expectation that one will occur.

The control of thirst is better understood, but there are some puzzling phenomena, one of which is known as "schedule-induced polydipsia". If a rat is

• *Hunger*, by J. Le Magnen, was published earlier this year by Cambridge University Press. The book is in the series *Problems in the Behavioural Sciences*, and is said to be "the first synthesis, by one hand, of the new knowledge on feeding behaviour". Price is hbk £20, \$34.50; pbk £7.95, \$14.95.

given access periodically (for example, once a minute) to a small amount of food in return for pressing a bar and if water is freely available, it may drink up to half its own body weight over a three-hour period. There is no satisfactory explanation for this maladaptive behaviour.

The picture Logue draws of the outcome of psychotherapy for the obese and for alcoholics is gloomy. There are two physiological reasons why slimming is so difficult. First, if you eat less food your metabolic rate goes down, so the rate of slimming is reduced. Second, obese people have an increased number of fat cells in their bodies: unfortunately, the number never decreases and there is evidence that the set-point for weight is set by the total number of fat cells. Hence anyone who was once fat but has succeeded in slimming may expect to be permanently hungry.

The only long-term solution appears to be by-pass surgery of the gastrointestinal tract, an operation which, surprisingly, appears to work not because less food is absorbed (the original rationale behind it), but because patients lose their liking for sweet substances and reduce their food intake. The patients apparently become more cheerful, but before you rush to the nearest surgical ward, you should note that the operation can cause liver disease and arthritis. Almost any treatment can reduce drinking — for a time; the recidivism rate is alarmingly high.

Not only are there no satisfactory treatments for obesity or alcoholism, we still do not know what causes them. It is possible that obesity can be produced by mothers who feed their babies whenever they are in distress, thus making them associate food with comfort. There is some evidence that there are hereditary factors at work in alcoholism. Oriental races are fortunate in that they feel dizzy and get palpitations on drinking, but the Japanese apparently put up with these feelings because their consumption of alcohol is rapidly increasing.

Recent work on eating and drinking has revealed the enormous complexity of the control mechanisms. Gone are the days when it was thought there were two different centres in the hypothalamus, one to turn hunger on and the other to turn it off. Hunger depends on a variety of brain circuits, and on several hormones (some probably still to be discovered) and neurotransmitters; moreover, the whole system is heavily affected by learning.

Logue's book is clear and is readily accessible to any scientist or even to the educated layman. There may be few solutions, but there are many interesting problems and ideas, and a mass of data that are worth pondering. □

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Formal assessment

Kathleen K. Smith

Functional Vertebrate Morphology. Edited by Milton Hildebrand, Dennis M. Bramble, Karel F. Liem and David B. Wake. *Harvard University Press*: 1985. Pp. 544. \$35, £29.75.

MILTON Hildebrand and his colleagues have compiled an excellent introduction to the literature, questions and approaches of functional vertebrate morphology. The book covers a large range of topics, and most vertebrate groups are discussed. Each chapter has been written by a leading worker, and although specialists may take exception to certain aspects of each one of them, the contributors do provide clear overviews of the issues involved.

In "Functional Adaptation in Skeletal Structures", for example, Lanyon and Rubin demonstrate the remarkable plasticity of bone, while in his article Goslow discusses the difficulty in disentangling various factors important in the neural control of locomotion. Much of the variation between chapters reflects their differing scope—topics addressed range from relatively narrow ones, such as "Digging of Quadrupeds" (Hildebrand) or "Terrestrial Locomotion Without Appendages" (Edwards), to immense ones such as "The Vertebrate Eye" (Levine) or "The Octavolateralis System" (Fay and Popper). These latter contributions, quite understandably, are not completely successful in summarizing their subject matter.

Some of the authors provide good, general overviews of an area (for example, Lauder on aquatic feeding in lower vertebrates, and Bramble and Wake on feeding mechanisms of lower tetrapods), whereas others, such as "Mastication, Food Transport and Swallowing" by Hiiemae and Crompton, are largely reviews of the work of one particular research group. A typical approach is to discuss the mechanical requirements of any activity, and then summarize the means by which animals meet them. Emerson ("Jumping and Leaping") and McNeill Alexander ("Body Support, Scaling and Allometry"), for example, provide quantitative analyses, predictions about animal form and finally tests of those predictions based on comparative data.

Only selected topics can be covered in a single volume dealing with such a large field, and the book is concerned almost entirely with the musculo-skeletal system; of the 18 chapters, 11 discuss locomotion and the axial skeleton, and 3 address feeding. This may in fact reflect vertebrate functional morphology as it is practised today, but a reader expecting a general introductory text might be surprised to

find so little on muscle, growth and development, neural systems, or internal organs or physiology.

An additional result of the selection of topics is a bias in approach. With chapters on such subjects as "Walking and Running" (Hildebrand), "Climbing" (Cartmill), "Swimming" (Webb and Blake), "Flying, Gliding and Soaring" (Norberg), "Ventilation" (Liem) and "Energetics and Locomotion" (Bennett) the emphasis is on tackling morphology by asking how animals solve particular mechanical problems. There is little sense of an organism and the multiple determinants of its form. Each chapter provides a clear analysis of its given topic; however, the organization of the book highlights the main dilemma facing evolutionary morphologists today—it is much easier to analyse a problem than an organism. The result, of course, has been the much-criticized reductionist approach to morphology.

This dilemma is illustrated by the

final chapter, "Morphology: Current Approaches and Concepts", by Liem and Wake. Here, "equilibrium analysis", which characterizes most of the contributions, is contrasted with an analysis of "intrinsic organizational features". This latter approach, which includes analyses of development, of phylogenetic history and of constraint, is currently the subject of much discussion, but receives little attention in the main portion of the text.

Functional Vertebrate Morphology is an outstanding summary of work in this field over the past 25 years and of the fruits of "equilibrium analysis". It is clear that success in the future will depend on the resolution of the differences between these two approaches, and the development of a specific and rigorous methodology for the study of the organism. □

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Back to the basics

John C. Brandt

Physics of Comets. By K.S. Krishna Swamy. *World Scientific/Wiley*: 1986. Pp. 273. £22, \$27.60.

COMET scientists are currently sorting through the bountiful harvest of data resulting from the direct observation of two comets. These comets, Halley and Giacobini-Zinner, have over the past year been probed directly, observed from rockets and spacecraft above the Earth and studied extensively by the networks of the International Halley Watch.

Thanks to these observations we now have a good idea of how the solar wind interacts with a comet to produce the large-scale plasma structures and the draped magnetic field. We now know that the spectacular disconnection events, where the entire plasma tail comes off the comet, are quite common. We have also learned that the molecular gas near the nucleus is composed primarily of water and that the principal minor constituents of this gas are carbon monoxide and carbon dioxide. The excellent images the spacecraft obtained of the nucleus reveal that it is shaped like a peanut or potato, has a very dark crust and emits material from sunward jets. More information will be available when results from a number of disciplines are brought together and compared.

Physics of Comets was completed before this recent burst of activity. The author's goal was to write a textbook that would discuss "the basic physical principles pertaining to various cometary phenomena", and the topics covered in-

clude the basic radiation laws, atomic and molecular spectroscopy, light scattering theory, vaporization theory and celestial mechanics. When appropriate, the cometary observations are described and the physics applied to the phenomena. For example, after a discussion of basic spectroscopy and cometary spectra, gas production rates are derived. Problems follow each chapter and an index is included.

Some of the descriptive material is now out of date (though the basic physics remains largely relevant) and there are a few questions concerning the balance. Mie scattering theory is extensively discussed, but although the theory applies to some of the dust in comets (possibly in the tails) other particles are very porous and irregularly shaped. This is suggested by the observation of negative polarization and by the appearance of cometary particles in the upper atmosphere. The author notes the limitations, but his emphasis on Mie theory may be inappropriate. The other area of concern is plasma physics. Some description of plasma phenomena in comets is given, but the physics necessary for interpretation is not developed. Comets are a rich source of such phenomena, and an accessible cosmic laboratory, and a complete understanding of them requires an understanding of their pervasive plasma phenomena.

The author has succeeded in producing a useful text that should be acquired by libraries and serious students of comets. The main problem with the book is timing. Those interested in current progress must look elsewhere. □

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The structure of the nucleon from deep inelastic lepton scattering and the nature of the strong interaction

T. Sloan*

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Results from deep inelastic neutrino and muon scattering experiments over a wide kinematic range provide quantitative evidence that the proton and neutron are composed of fractionally charged quarks bound together by gluons. These and other data also provide a test of quantum chromodynamics (QCD), the currently favoured theory of the strong interaction between quarks.

OVER the past two decades it has become generally accepted that all the strongly interacting particles (hadrons) observed in the laboratory are composed of elementary objects known as quarks. This concept originated from the study of the symmetries in the quantum numbers of the hadrons¹, and was reinforced by observations of deep inelastic electron and neutrino scattering², and later by e^+e^- annihilation experiments³. Quantitative tests of this picture have been permitted by the advent of data covering a wide kinematic range, from deep inelastic muon and neutrino scattering experiments. These experiments observed the scattering of beams of muons and neutrinos from targets made of the most commonly occurring hadrons, namely protons and neutrons (nucleons).

The quark model of hadrons

Applying the original ideas of Gell-Mann *et al.*¹, the variety of light hadrons can be explained in terms of different combinations of two types or 'flavours' of quarks which are labelled u and d (for up and down). To explain the properties of heavier particles, further quantum numbers (strangeness, charm and beauty) must be assigned to them, associated with quark flavours s, c and b, respectively. A further flavour, t, is predicted from the symmetry arguments but has not yet been confirmed. To explain simultaneously the symmetries and the charges of the observed hadrons the quarks are required to have an electric charge which is a fraction of the electronic charge, e . Thus the u and d quarks should have charges $2/3e$ and $-1/3e$, respectively. In this picture the proton consists of 2 u quarks and 1 d quark and the neutron consists of 2 d quarks and 1 u quark. (See ref. 4 for a review of the quark model).

The existence of strongly interacting particle with spin 1/2 implies that the quarks also have half-integral spins; that is, they are fermions. The wave functions of identical quarks in such particles should be antisymmetric under interchange, and for this it is necessary to postulate that the quarks carry an extra quantum number, termed 'colour'. The quarks can carry three possible colours, and hadrons consist of a mixture of colours, such that they are colourless. The colour quantum number of each quark acts as the counterpart of charge in electromagnetism for the strong force between the quarks. This force is generated by the exchange of gluons, the quanta of the strong interaction, between the colour charges of the quarks. In this picture a hadron consists of its basic building blocks, the 'valence' quarks, in a sea of exchanged gluons. Virtual quark-antiquark pairs are produced from the gluons, giving rise to 'sea' quarks.

The quarks can also interact through the weak nuclear interaction, by the exchange of W^+ and Z^0 vector bosons, which have

recently been observed directly^{5,6}. As quarks are electrically charged, they also interact electromagnetically by the exchange of virtual photons, the quanta of the electromagnetic field. These interactions allow the quark structure of the nucleon to be probed using beams of leptons, which interact only weakly (neutrinos) or electromagnetically (electrons and muons; see Fig. 1). The cross-sections for such interactions are readily calculable and, as the leptons can be assumed to be point-like, these experiments allow the measurement of the structure of the target nucleon. The point-like nature of the charged leptons has been verified by comparing high-precision measurements with calculations of their gyromagnetic ratios⁷.

Quantum chromodynamics (QCD) is the currently favoured theory of the strong interaction between the quarks. In this theory⁸, as explained above, the forces between the quarks are produced by the exchange of gluons between their colour charges. The gluons are massless, with spin 1 and negative parity. The strength of the field between a quark and a gluon is parameterized by the coupling constant of the theory, α_s , which is a dimensionless quantity. The vacuum polarization contributions cause this coupling constant (or interaction strength) to decrease as the energy scale, Q^2 , increases⁹. An approximate ('leading log') expression for the coupling constant is

$$\alpha_s(Q^2) = \frac{12\pi}{(33 - 2n_f) \ln(Q^2/\Lambda^2)} \quad (1)$$

where n_f is the number of quark flavours that can be produced at the energy of the interaction and Λ is a free parameter which must be determined experimentally. It can be seen from equation (1) that at large energy scales (Q^2), which are equivalent to short distances, the coupling constant decreases and perturbation theory can be used to calculate hadronic processes. The increase of the coupling constant at large distances (small Q^2) is thought to confine the quarks and gluons within a hadron, so that they have never been reproducibly observed as free particles in the laboratory¹⁰.

The strong interaction between quarks as described by QCD is simple. The more complicated forces between hadrons, which are responsible for the binding of nucleons in nuclei, are thought to be generated by multiple quark interactions. Here I will discuss the evidence for the quark structure of the nucleon, and describe some tests of the QCD theory.

Deep inelastic lepton scattering

The interaction between the lepton and the nucleon can be represented by the Feynman graph shown in Fig. 1. The incoming muon or electron (or neutrino or antineutrino) exchanges a virtual photon (or a virtual W or Z boson). It is these virtual

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particles which probe the structure of the nucleon as they are absorbed by its constituent parts. The wavelength of the probe is much shorter than the size of the nucleon; it can therefore be used to examine the nucleon's structure. The term 'deep inelastic scattering', applied to these experiments, refers to this ability to probe deeply into the nucleon.

Applying the Feynman rules to the graph in Fig. 1, the cross-sections for deep inelastic scattering from unpolarized target nucleons can be written as follows¹¹:

$$\frac{d^2\sigma^{\mu N}}{dx dy} = \frac{8\pi\alpha^2 ME}{Q^4} \left[y^2 x F_1^{\mu N}(x, Q^2) + \left(1 - y - \frac{Mxy}{2E}\right) F_2^{\mu N}(x, Q^2) \right]$$

$$\frac{d^2\sigma^{\nu, (\bar{\nu})N}}{dx dy} = \frac{G^2 ME}{\pi} \left[y^2 x F_1^{\nu N}(x, Q^2) + \left(1 - y - \frac{Mxy}{2E}\right) F_2^{\nu N}(x, Q^2) + y \left(1 - \frac{y}{2}\right) x F_3^{\nu N}(x, Q^2) \right] \quad (2)$$

where E and E' are the energy of the incident lepton and the lepton scattered at angle θ , M is the mass of the nucleon, $\nu = E - E'$ is the energy of the exchanged virtual photon or boson, $Q^2 (=4EE'\sin^2\theta/2)$ is the negative squared four-momentum transferred to the virtual photon, $y = \nu/E$ and $x = Q^2/2M\nu$. In the case of incident charged leptons (μ and e) the electromagnetic coupling constant, $\alpha = 1/137$, enters equations (2). This is replaced for neutrinos by the weak interaction coupling constant, G , measured in nuclear β -decay. The functions $F_1^{\mu N}$, $F_2^{\mu N}$, $F_1^{\nu N}$, $F_2^{\nu N}$ and $F_3^{\nu N}$ are structure functions which are not necessarily the same in neutrino and muon scattering. The observation of consistency between the structure functions measured in muon and neutrino experiments proves that each scatters off the same constituents of the nucleon (see below).

As the exchanged photon or vector boson is virtual, it can have a mass which is $-Q^2$. In the case of the photon, this is allowed by the uncertainty principle, as it exists for such a short time. Similarly it can have a longitudinal as well as transverse polarization. In contrast, real photons have zero mass and pure transverse polarization. The wavelength of the virtual photon in muon or electron scattering is

$$\lambda = \frac{h}{p} = \frac{hc}{(\nu^2 + Q^2)^{1/2}} \approx \frac{2Mx}{Q^2} (hc) \quad (3)$$

where h and c are Planck's constant and the speed of light, respectively. Thus at fixed x the wavelength (which is the minimum length scale that can be probed) is inversely proportional to Q^2 .

The structure functions F_1 , F_2 and F_3 are proportional to the absorption cross-sections for the different polarization states of the exchanged virtual photon or vector boson¹². They are related to the probability of the absorption of the exchanged particle by the target nucleon, and depend on the distribution of matter in the nucleon, as explained below. For charged leptons only two structure functions are required, due to the conservation of parity in the electromagnetic interaction. In contrast, for neutrinos a third structure function enters as the weak interaction is parity violating and is a mixture of vector and axial vector currents, which are of opposite parity. The interference between these amplitudes of opposite parity introduces the third structure function, $F_3^{\nu N}$. These three structure functions have been measured in deep inelastic scattering. The simple picture in equations (2) and Fig. 1 is complicated by the need to make corrections for higher-order processes. However, such radiative corrections are at the level of a few per cent and can be routinely made^{13,14}.

The first measurements of the structure functions $F_1^{\mu N}$ and $F_2^{\mu N}$ were made at SLAC^{15,16} (the Stanford Linear Accelerator

Lepton in	Lepton out	Interaction	Exchanged boson
$\mu^\pm$	$\mu^\pm$	Electromagnetic	Photon
$e^\pm$	$e^\pm$	Weak	W^+
ν_μ	μ^-	Charged current	W^-
$\bar{\nu}_\mu$	μ^+	Weak	Z^0
ν_μ	ν_μ	Neutral current	
$\bar{\nu}_\mu$	$\bar{\nu}_\mu$		

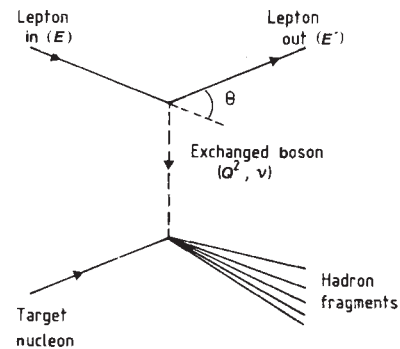


Fig. 1 Lowest-order Feynman graph describing deep inelastic lepton-nucleon scattering.

Center) and DESY¹⁷ (Deutsches Elektronen-Synchrotron, Hamburg). More extensive measurements have been made recently in neutrino¹⁸ and muon¹⁹⁻²⁴ experiments; a sample of the data is shown in Fig. 2. This shows the measurements of the structure function $F_2^{\mu N}$ by the European muon collaboration (EMC) at CERN²⁰ (Centre Européen pour la Recherche Nucléaire), plotted as a function of Q^2 at fixed values of x . The main feature of the data is that the structure function is approximately independent of Q^2 at fixed x .

Figure 2 lists some typical values of the wavelength of the virtual photon. The absorption probability of the virtual photon is approximately independent of the photon wavelength, and hence of any length scale. In other words, the interaction probability is independent of the size of the region of the nucleon sampled by the virtual photon. Such behaviour, known as scaling, requires the presence of point-like electric charges within the nucleon, as the photon is absorbed by charged objects.

Such point-like objects were first named partons by Feynman². In the following it is shown that they can be identified with the fractionally charged quarks postulated to explain the symmetry of the observed spectrum of the hadrons¹. Scaling was first predicted, using these symmetry arguments, by Bjorken²⁵, and was observed experimentally in the early deep inelastic electron scattering experiments¹⁵⁻¹⁷.

Evidence that partons are quarks

A simple kinematic argument can be used to show that the variable $x = Q^2/2M\nu$ is the fraction of the momentum of the nucleon carried by each parton. In this picture, neglecting the small variations of the structure functions with Q^2 , which will be discussed below, it can be shown²⁶ that for the nucleon (that is, for the average of the scattering from a proton and a neutron),

$$2xF_1^{\nu N}(x) = F_2^{\nu N}(x) = \sum_f x[q_f(x) + \bar{q}_f(x)]$$

$$xF_3^{\nu N}(x) = \sum_f x[q_f(x) - \bar{q}_f(x)] \quad (4)$$

$$2xF_1^{\mu N}(x) = F_2^{\mu N}(x) = \sum_f e_f^2 x[q_f(x) + \bar{q}_f(x)]$$

where $q_f(x)$ is the probability that a parton of flavour f and charge e_f (in units of the electronic charge) carries a fraction x of the momentum of the nucleon, and $\bar{q}$ is that for an antiparton. The structure functions for neutrinos and muons will be related through equations (4) if the neutrinos and muons interact with the same substructures in the nucleon. The extra factor e_f^2 in

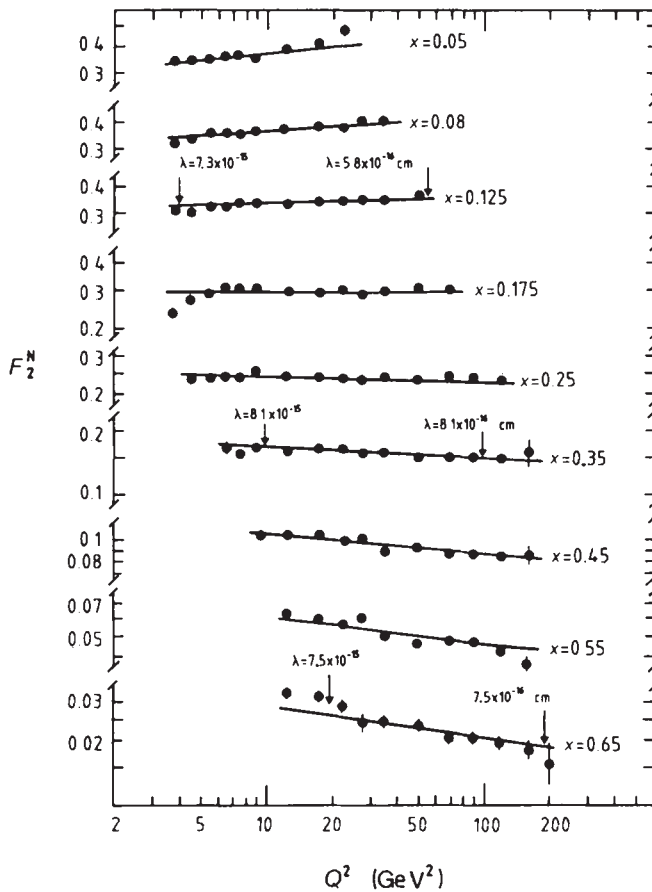


Fig. 2 Nucleon structure function, F_2^N , measured in iron by the EMC group¹⁹. The points show the measurements; the curves are the QCD fits to the data, described in the text. Typical values of the virtual photon wavelength, λ , are shown.

the case of muon scattering enters because the virtual photon couples to the charge of the struck parton. Comparisons between the structure functions F_2 measured in muon and neutrino experiments may be used to test the consistency of this picture and deduce the charges on the partons.

It is assumed from the symmetry arguments applied to the spectrum of light hadrons¹ that the nucleon contains two valence parton types, each with electric charges e_u and e_d . These parton types must have electric charges of opposite sign; otherwise, electrically neutral hadrons such as the neutron would not exist. Furthermore, as the strong interaction properties of the proton and neutron are identical and they differ only in their electromagnetic properties, the distribution of positively charged partons in the proton must be the same as that of negative partons in the neutron, and vice versa. This is the principal of isospin symmetry as applied to the nucleon. It then follows from equations (4) that

$$\frac{F_2^{vN}}{F_2^{pN}} = \frac{\frac{1}{2}(F_2^{vp} + F_2^{vn})}{\frac{1}{2}(F_2^{pp} + F_2^{pn})} = \frac{1}{2}(e_u^2 + e_d^2) \quad (5)$$

neglecting small corrections for the strange and charmed parton content of the nucleon sea. Figure 3 shows the ratio $(F_2^{vN}/F_2^{pN}) \times \frac{5}{18}$ as a function of x from the neutrino^{27,28} and muon²⁰ data, where the factor $\frac{5}{18}$ is the expected value of the mean square charges of the partons, assuming $\frac{2}{3}$ and $\frac{1}{3}$ for the u and d parton charges, respectively. For such fractionally charged partons, equations (4) and (5) indicate that the measured ratio should be independent of x and consistent with unity, even though the individual structure functions vary strongly with x (see Fig. 2). The measured ratios shown in Fig. 3 are consistent with this behaviour within the experimental errors if overall normaliz-

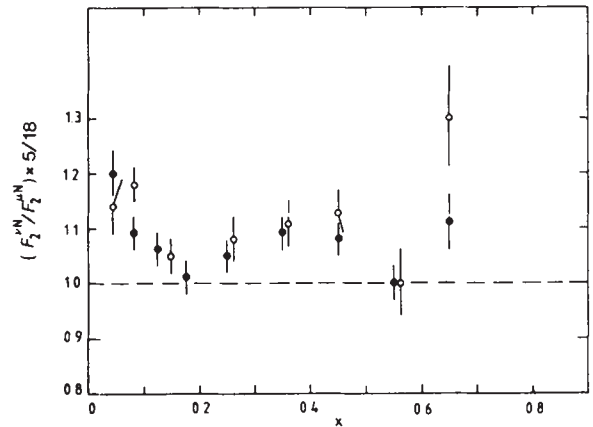


Fig. 3 Ratio $(\times \frac{5}{18})$ of the nucleon structure functions from neutrino^{27,28} and muon²⁰ data as a function of x for scattering from an iron target. Small corrections ($\sim 5\%$) for the charm and strange content of the nucleon sea^{40,41} have been applied to the measured ratios at $x < 0.2$.

ation shifts of 5–10% are applied to the data. These are within the quoted normalization uncertainties of the muon and neutrino experiments.

The agreement between the data and the predictions of equations (4) shows that the structure functions measured in neutrino and muon scattering are consistent with each other, proving that the lepton probes are scattering from the same point-like nucleon constituents.

The neutrino data can be used to check the number of valence partons in the nucleon. From equations (4), $x F_3$ is related to the difference between the parton and antiparton distributions in the nucleon. Assuming that the contributions from the sea of partons and antipartons is the same, the sea distributions cancel, yielding

$$x F_3 = \sum_v x q_v(x)$$

Thus,

$$\int_0^1 F_3 dx = \int_0^1 \sum_v q_v(x) (1 - \alpha_s/\pi) dx \quad (6)$$

represents the total number of valence partons (denoted by subscript v) in the nucleon, apart from the small correction factor (α_s/π) , which arises from QCD effects²⁹. This relationship is known as the Gross-Llewellyn-Smith sum rule³⁰. The measured value of the integral on the left-hand side of equation (6) is 2.81 ± 0.16 (ref. 31). Applying the correction factor, with $\alpha_s = 0.2$ (see below), gives the total number of valence partons to be 3.0 ± 0.2 .

Similarly, the integral relationship known as the Adler sum rule³² also follows from equations (4):

$$\int_0^1 \frac{F_2^{vN} - F_2^{pN}}{x} dx = \int_0^1 u_v(x) - d_v(x) dx \quad (7)$$

where the assumptions are made that the sea contributions in the proton and neutron are the same. The right-hand side of equation (7) represents the difference between the total number of u and d valence partons in the proton. Deducing the left-hand side from the data of the WA25 collaboration³³, its mean value is 1.07 ± 0.10 .

From the sum (equation (6)) and the difference (equation (7)), the number of u valence partons in the proton is found to be 2.1 ± 0.1 and the number of d valence partons is 1.0 ± 0.1 . These measured values are consistent with the values of two u partons and one d parton in the proton expected from the symmetries in the hadron spectrum¹.

To derive the charges on the partons, we make the same assumptions as above. Applying equations (4), the difference

between the neutron and proton structure functions for muon scattering is

$$F_2^{\mu p} - F_2^{\mu n} = (e_u^2 - e_d^2)(xu_v(x) - xd_v(x)) \quad (8)$$

From equation (8), taking two u-flavoured partons and one d-flavour in the proton (that is, $\int u_v dx = 2$ and $\int d_v dx = 1$), it follows that

$$I \equiv \int_0^1 \frac{F_2^{\mu p} - F_2^{\mu n}}{x} dx = e_u^2 - e_d^2 \quad (9)$$

Figure 4 shows the variation of $F_2^{\mu p} - F_2^{\mu n}$ measured from deep inelastic muon scattering from hydrogen¹⁹ and deuterium²¹ targets. Calculating the integral from the data gives

$$I = 0.25 \pm 0.12 \quad (10)$$

Note that evaluation of these integrals involves extrapolation of the data to $x = 0$, where measurements do not exist. A large contribution to the quoted error comes from these extrapolations. It is assumed that the structure functions are well behaved and approach zero as x tends to zero.

The mean value of the ratio $F_2^{\mu n}/F_2^{\mu p}$ from Fig. 3 is (0.29 ± 0.02) , which is half the sum of the squared charges on the partons. The integral in equation (10) gives the difference of the squared charges of the partons. Solving for the individual parton charges gives

$$|e_u| = 0.64 \pm 0.05; |e_d| = 0.41 \pm 0.08 \quad (11)$$

which are consistent, within the experimental uncertainties, with the values of $\frac{2}{3}$ and $\frac{1}{3}$ predicted for quarks.

These measurements thus show quantitatively that neutrinos and muons each scatter from the same point-like nucleon constituents (partons) of charge $\frac{2}{3}$ and $\frac{1}{3}$. The basic building blocks (the valence partons) of the proton consist of 2 u-flavoured and a single d-flavoured parton. This gives direct experimental proof that the point-like objects in the nucleon are the same as the quarks proposed from the symmetry arguments¹, and verifies clearly the quark model of the nucleon.

Evidence for gluons

The evidence for gluons is more qualitative than that for quarks. However, there are many phenomena which are explained by such objects thus the circumstantial evidence for them is strong. Some of these phenomena are described below.

Integrals of F_2 in muon and neutrino scattering. The integral of F_2 in deep inelastic scattering is related to the total fraction of the momentum of the nucleon carried by the charged constituents, that is, the quarks. It can be seen from equations (4) that

$$\begin{aligned} I &= \int_0^1 F_2^{\mu N} dx \\ &= \int_0^1 \frac{1}{2}(F_2^{\mu p} + F_2^{\mu n}) dx \\ &= \int_0^1 \frac{1}{2}(e_u^2 + e_d^2) \sum_f xq_f(x) dx - \frac{1}{6} \int_0^1 x(2s - 2c) dx \end{aligned} \quad (12)$$

The last term arises from the presence of strange and charmed quarks in the nucleon sea and represents a small correction which is $\sim 3\%$ of the integral. The EMC data on iron²⁰ gave $I = 0.132 \pm 0.010$ at $Q^2 = 15 \text{ GeV}^2$. The integral $\sum_f \int_0^1 xq_f(x) dx$ represents the total momentum fraction of the nucleon carried by quarks, which is $\frac{18}{5} \times 0.132 = 0.49 \pm 0.03$.

The same integral measured in deep inelastic neutrino scattering measures the momentum fraction of the quarks directly, without the need for correction for the charge of the quarks. The neutrino data¹⁸ show the same values for this momentum fraction, within experimental errors.

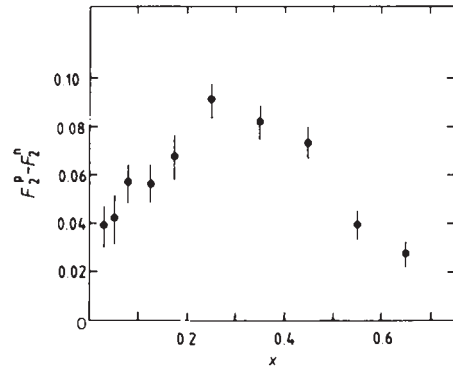


Fig. 4 Measured difference between the proton and neutron structure functions as a function of x in deep inelastic muon scattering on hydrogen¹⁹ and deuterium²¹.

These results show that only about half the momentum of the nucleon is carried by quarks. The remaining momentum must be carried by objects which do not interact either electromagnetically or weakly, otherwise the integrals would be different for neutrino and muon scattering. Thus some other constituent of the nucleon exists which carries momentum but does not interact weakly or electromagnetically. Gluons, which are the quanta of the strong interaction, fulfil these conditions.

Deviations from scaling of the nucleon structure functions. All measurements show that, although the nucleon structure functions at fixed x are approximately independent of Q^2 , they indicate a similar pattern of scaling violations. This can be seen in Fig. 2, which shows that the structure functions tend to increase with increasing Q^2 at small x , and decrease with increasing Q^2 at large x . If this behaviour were due to a substructure of the quarks, the structure functions would change in the same way with Q^2 at all values of x , since as Q^2 increases the resolution of the substructure would improve.

Figure 5 shows that the existence of gluons explains the behaviour of the data. Gluons can be emitted from the quark by the strong interaction process, a phenomenon analogous to bremsstrahlung from accelerated electric charges. At fixed x ($= Q^2/2M\nu$), as Q^2 increases, so does the energy of the virtual boson, ν . The probability of such bremsstrahlung increases with energy ν , as a wider range of transverse momenta is probed in the parton cloud of the target. Thus, as explained in Fig. 5, at higher values of Q^2 more bremsstrahlung occurs, leading to a softening of the quark distribution functions and hence of F_2 (equations (4)). Thus quarks from a high-momentum fraction, x , tend to be scattered at a lower value of x .

Three-jet events in e^+e^- annihilation. Perhaps the most direct positive evidence for gluons comes from the observation of jet-like structures in high-energy e^+e^- annihilation experiments³. Colliding beams of e^+ and e^- particles have been observed to annihilate into hadrons, most of which are found to form two jet-like clusters. This is interpreted according to the Feynman graph shown in Fig. 6a. The e^+ and e^- annihilate into a single virtual photon, which then couples to a $q\bar{q}$ pair, forming two jet clusters emitted in opposite directions to conserve momentum. Some events are found to contain a third jet, which is thought to be produced by the emission of a gluon (Fig. 6b).

Tests of QCD and measurement of α_s

In principle, all processes involving the interaction of hadrons should provide testing grounds for QCD. In practice the tests³⁴ must be restricted to those processes for which reliable calculations can be made. Such calculations invariably involve the use of perturbation theory. Thus for any interaction which has subprocesses with low energy transfers, incurring large values

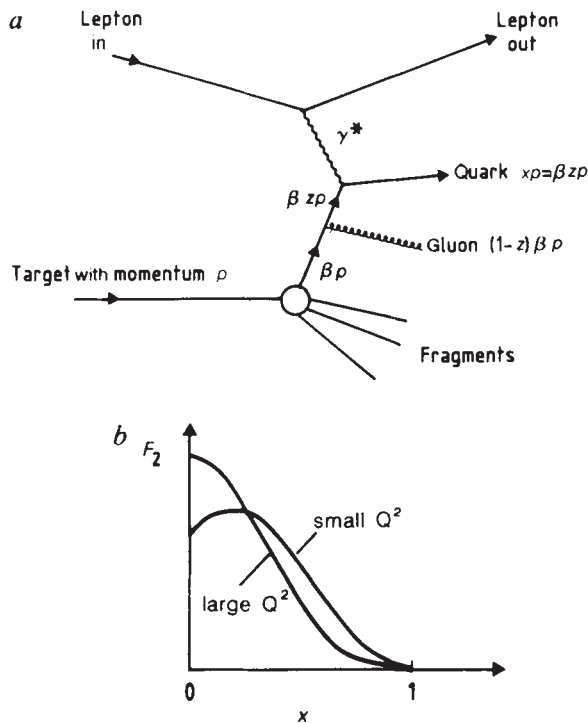


Fig. 5 *a*, Softening of a scattered quark by gluon bremsstrahlung emission. The quark carrying a momentum fraction β of the target emits a gluon of momentum fraction $(1-z)$ of the quark's momentum. The momentum fraction of the quark scattered by the virtual photon is measured as $x = \beta z$ ($= Q^2/2M\nu$), whereas its original momentum fraction was β . The result of such processes (*b*) is that quarks at large x tend to be lost at larger Q^2 and reappear at smaller values of x .

of α_s (equation (1)), reliable calculations cannot be made. Such interactions are called non-perturbative processes.

Several processes exist at energy scales that are sufficiently large for perturbation theory to be applied. From these processes QCD can be tested, by comparing the predictions of the theory with the measurements and obtaining a value of the strong coupling constant α_s from the comparison. QCD can be deemed to have passed the test if a consistent set of values of α_s are obtained which vary with energy according to the form given in equation (1). The measurements of α_s from the experimental tests are shown as a function of Q^2 in Fig. 7 and will be described below. The curves in Fig. 7 show the variation of α_s expected from equation (1).

Deep inelastic scattering provides one test of QCD. The curves in Fig. 2 show quantitative fits of the data to the processes used to explain the scaling violations described above. These curves show that QCD gives a reasonable representation over the whole range of the data^{19,20}. The values of α_s averaged over the neutrino and muon data are labelled DIS in Fig. 7. Some theoretical uncertainty arises from the effects of coherent interactions between the quarks ('high twist' effects). These were found to have little effect on the determination of α_s at the large values of Q^2 relevant to the muon data¹⁹, and it is assumed that they also have little effect for the neutrino data.

Measurements of α_s have been made in e^+e^- annihilation experiments. The value measured from the total cross-section for annihilation to hadrons is the one which has least theoretical uncertainty³⁴. The ratio of the total cross-section for annihilation to hadrons to that for annihilation to muons is given by

$$R = \frac{\sigma(e^+e^- \rightarrow \text{hadrons})}{\sigma(e^+e^- \rightarrow \mu^+\mu^-)} = 3 \sum_i e_i^2 (1 + \alpha_s/\pi) \quad (13)$$

This ratio can be understood from Fig. 6a. The hadrons are produced by adding the contributions of all possible flavours

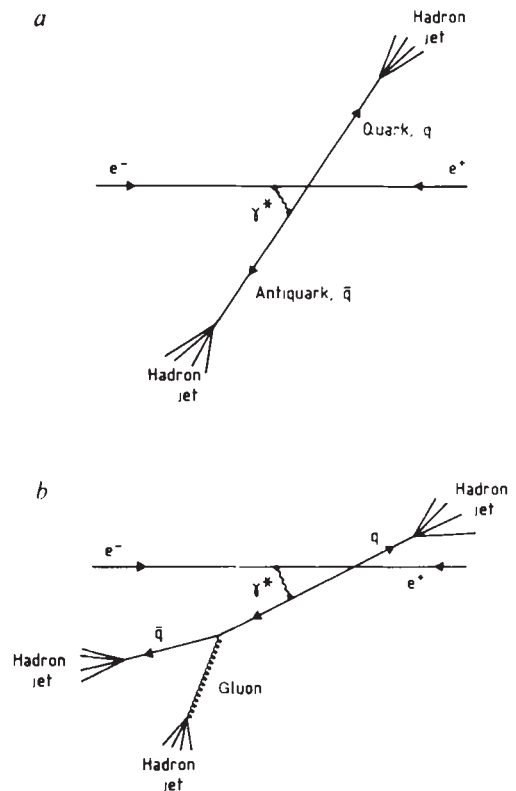


Fig. 6 Feynman diagram showing the process used to explain the observation of two-jet (*a*) and three-jet (*b*) events in high-energy e^+e^- annihilation. The exchanged virtual photon carries energy but zero momentum.

of quark pairs. In taking the ratio in equation (13), all factors cancel except the coupling of the virtual photon to the charges on the quarks. The factor 3 represents the fact that the quarks can be produced with three different colours and the factor $(1 + \alpha_s/\pi)$ is a correction for the terms involving gluons²⁹. The spectacular success of this simple expression in reproducing the measured values of R gives further strong evidence for quarks. Encouraged by this success, the measurements can be used to obtain the small correction term α_s/π . The value obtained, averaging over all experiments, is $\alpha_s(Q^2 = 1,200 \text{ GeV}^2) = 0.19 \pm 0.06$, where the error is purely experimental. This point is labelled $R(e^+e^-)$ in Fig. 7.

The value of α_s has also been measured from the event shapes observed in e^+e^- annihilations at a centre-of-mass energy of $\sim 30 \text{ GeV}$. In principle, the ratio of the relative numbers of three-jet and two-jet events can be used to derive α_s , due to the coupling of the gluon to the quarks (Fig. 6b). In practice, as the gluon is produced by a bremsstrahlung process, it is usually emitted at a relatively small angle to the quark and the separation of three-jet from two-jet events is often uncertain. The data were analysed in kinematic regions where such separation is good, and corrections for losses were made using models for the production of hadrons from quarks (fragmentation). The average value from all measurements³⁴ was $\alpha_s(Q^2 \approx 1,000 \text{ GeV}^2) = 0.16 \pm 0.03$, where the error shows the effect of using different models of the fragmentation process. This point is labelled ' e^+e^- shapes' in Fig. 7.

Jet production has been observed by the UA1 and UA2 collaborations at the CERN $p\bar{p}$ collider at a centre-of-mass energy of 630 GeV . The jets are much more clearly separated at the higher energies of this accelerator than at the lower-energy e^+e^- machines. The collisions between protons and antiprotons are more complicated than e^+e^- annihilation, as they involve gluon-gluon, quark-gluon and quark-antiquark interactions.

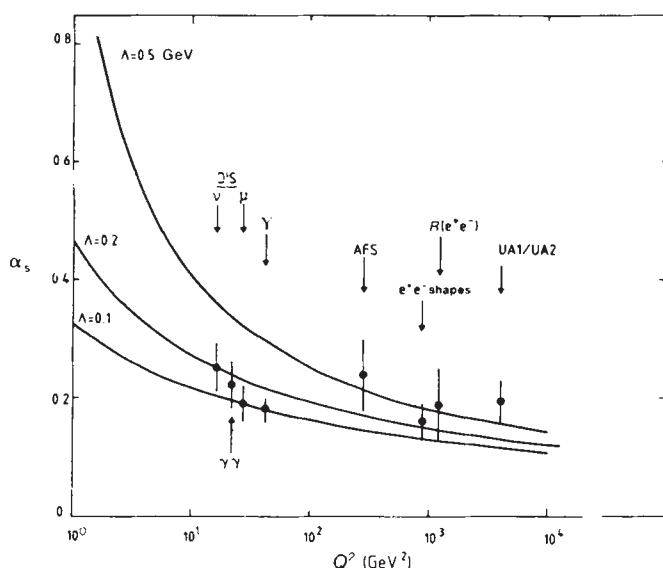


Fig. 7 Measured values of α_s from all techniques, as a function of Q^2 . The curves show the values computed from equation (1), with $n_f = 4$. Labelled points are discussed in the text.

All of these can produce extra gluons by bremsstrahlung, and the events with three jets are well separated from those with two jets. The ratio of the number of three-jet to two-jet events can be measured without ambiguity due to overlap, and this provides a measure of α_s . Some theoretical uncertainties occur due to non-scaling effects and higher-order effects. In addition, the Q^2 scale is uncertain and may be different for the two-jet and three-jet events. The average value obtained^{35,36} from these experiments (labelled UA1/UA2 in Fig. 7) is $\alpha_s(Q^2 = 4,000 \text{ GeV}^2) = 0.19 \pm 0.04$. A similar technique has been applied to p-p data at the lower centre-of-mass energy of 63 GeV, taken at the CERN intersecting storage ring (ISR). At such low energies the jets are less distinct and an analysis similar to that performed for the e^+e^- experiments must be made. This yielded $\alpha_s(Q^2 = 225 \text{ GeV}^2) = 0.24 \pm 0.06$ (ref. 37). This point is labelled AFS in Fig. 7.

The strong coupling constant has also been measured in the decay of the mesons in the Y family. These are heavy, spin-1 resonances formed from $b\bar{b}$ quark pairs. They are thought to decay predominantly by annihilating to three gluons which form daughter hadrons. The branching ratio for the decay to a photon and hadrons has been measured. The ratio of this mode to the decay to hadrons only is proportional to the ratio of the electromagnetic coupling constant ($\alpha = \frac{1}{137}$) to α_s , because a gluon is replaced by a photon with the same quantum numbers. Similarly, α_s can be obtained by comparing the branching ratios for $Y \rightarrow \mu^+\mu^-$ to $Y \rightarrow \text{hadrons}$, for which, in the former, the three

gluons are replaced by three virtual photons. Hence the ratio of the branching ratios for the two processes will be inversely proportional to α_s^3 (ref. 34). The values of α_s obtained from these two methods are (0.20 ± 0.03) and (0.17 ± 0.04) , respectively. The higher-order terms in the perturbation expansion are rather large and depend on the renormalization scheme of the theory. These terms were neglected in deducing the values of α_s , and the quoted errors are the changes obtained when different renormalization schemes are used to compute such terms. Also, the Q^2 for these measurements is uncertain and could lie anywhere between $(0.16M)^2$ to M^2 (ref. 37), where M is the mass of the Y meson. This is a somewhat conservative approach, and smaller errors on α_s have been quoted for this measurement^{34,37}. The mean of these two measurements is plotted in Fig. 7 at $Q^2 = M^2/2$ and labelled Y.

A measurement of α_s has been made by studying photon-photon collisions in the e^+e^- scattering experiments. In these experiments a virtual photon at high Q^2 from one of the leptons scatters from a virtual photon from the second lepton at much lower Q^2 . The lepton producing the high- Q^2 photon is detected in the apparatus. To extract a value of α_s , the data must be compared to models which predict the observed yield from several different contributions³⁸. Some uncertainty exists in these models; nevertheless, a value of α_s has been obtained and this point is labelled $\gamma\gamma$ in Fig. 7.

Figure 7 shows that the widely diverse phenomena described above give a consistent set of values of α_s . This demonstrates the success of QCD as the theory of strong interactions. The curves in Fig. 7 represent the expected variation of α_s with Q^2 (from equation (1)). Within the experimental and theoretical uncertainties it cannot be proven that α_s shows the expected variation with Q^2 ; however, the data are not inconsistent with such a variation. More precise experiments, free from theoretical uncertainties, will be required to establish this point.

The success of QCD shows that the strong interaction between the quarks is basically simple. In contrast, the hadron-hadron interactions are more complex, as they involve multi-body interactions between quarks. Unfortunately, reliable calculations can be made only where perturbation theory can be applied. Mathematical techniques are being developed, involving the use of Monte Carlo methods, which could allow the distance scale of the theory to be extended³⁹. The perfection of these techniques will allow more stringent tests to be made at lower values of Q^2 , which should provide better measurements of the variation of α_s with Q^2 . If these attempts are successful they will allow the masses of the hadrons to be calculated, and perhaps more complicated processes such as nuclear binding.

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Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor

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*Cloning and sequence analysis of DNA complementary to porcine cerebral messenger RNA encoding the muscarinic acetylcholine receptor predict the complete amino-acid sequence of this protein. Expression of the complementary DNA produces functional muscarinic receptor in *Xenopus* oocytes. The muscarinic receptor is homologous with the β -adrenergic receptor and rhodopsin in both amino-acid sequence and suggested transmembrane topography.*

THE muscarinic acetylcholine receptor (mAChR) mediates various cellular responses, including inhibition of adenylate cyclase, breakdown of phosphoinositides and modulation of potassium channels through the action of guanine nucleotide-binding regulatory proteins (G proteins)¹⁻³. Are there different types of mAChR, each responsible for one of its many biochemical and physiological effects? Pharmacologically distinguishable subtypes of the mAChR occur in different tissues, and the substantial difference in apparent affinity for the antagonist pirenzepine observed for mAChRs from various loci has led to a provisional classification into M₁ and M₂ subtypes⁴⁻⁶. But it is not known whether these subtypes reflect a structural difference of the mAChR protein resulting from translation of distinct messenger RNAs or from post-translational modifications, or whether they are caused by an environmental difference such as interaction with other molecules. The mAChR from porcine cerebrum has been purified to apparent homogeneity by affinity chromatography, and its relative molecular mass (M_r) estimated to be ~70,000 (ref. 7). The purified mAChR, when reconstituted in phospholipid vesicles together with a purified G protein (G_i or G_o), is functional in that the receptor/G-protein complex exhibits guanine nucleotide-sensitive high affinity agonist binding and agonist-stimulated GTPase activity^{8,9}.

One approach to elucidation of the molecular basis of the functional heterogeneity of the mAChR and the mechanisms underlying the multiple muscarinic responses is to clone complementary DNA for the receptor and to study the functional properties of the receptor generated by expression of the cDNA. Here we describe the isolation of cDNA clones derived from porcine cerebral mRNA encoding the mAChR. Nucleotide sequence analysis of the cDNA reveals the complete amino-acid sequence of the receptor. We have expressed the cDNA to produce functional mAChR in *Xenopus* oocytes, and find that the tissue location of the RNA hybridizing with the cDNA, as well as the high apparent binding affinity for pirenzepine of the mAChR expressed from the cDNA, is consistent with the notion that the cDNA encodes the mAChR of the M₁ subtype.

Isolation of cDNA

The approach used to clone cDNA for the mAChR was to screen a library of cDNA clones by hybridization with

oligodeoxyribonucleotide probes synthesized on the basis of partial amino-acid sequence data. The mAChR was solubilized from porcine cerebrum and purified as described previously⁷. The purified protein was digested with trypsin and the resulting peptides fractionated by reverse-phase HPLC (Fig. 1A). Three peptides (I, II and III) were isolated and analysed for amino-acid sequence with the use of a gas-phase sequencer¹⁰ (Fig. 1B).

A library of cDNA clones derived from poly(A)⁺ RNA from porcine cerebrum was constructed using the plasmid DNA vector of Okayama and Berg¹¹. The cDNA library was screened by hybridization with an oligodeoxyribonucleotide probe containing all possible cDNA sequences predicted from the partial amino-acid sequence Met-Pro-Met-Val-Asp of the tryptic peptide II (excluding the third position of the aspartic acid codon). Six hybridizable clones were isolated from ~7 × 10⁵ transformants. Three of them (pmACR34, pmACR37 and pmACR40) hybridize with both of two longer cDNA probes designed on the basis of frequent codon choices¹² for the 13-amino-acid sequence determined for the tryptic peptide II and the carboxy-terminal 8-amino-acid sequence determined for the tryptic peptide I. Partial nucleotide sequence analysis¹³ of clones pmACR34 and pmACR37 reveals that they carry coding sequences for the entire amino-acid sequences determined for the tryptic peptides I and II. Further screening of the same cDNA library with a 1,557-base pair (bp) probe derived from clone pmACR34 yielded 27 hybridizable clones from ~2 × 10⁶ transformants. Two of them (clones pmACR84 and pmACR60), that apparently carry the largest cDNA inserts, were analysed for nucleotide sequence by the procedure of Maxam and Gilbert¹³ according to the strategy indicated in Fig. 2a. Experimental details for the isolation of the cDNA clones are given in Fig. 2 legend.

Protein sequence

Figure 2b shows the 2,884-nucleotide sequence (excluding the poly(dA) tract) of the cDNA encoding the mAChR from porcine cerebrum, determined using clones pmACR84 (nucleotides -443 to 2,441) and pmACR60 (nucleotides -171 to 2,441). There is no difference between the nucleotides in the cDNA sequences of the two clones. All three partial amino-acid sequences determined (see Fig. 1B) are encoded by the cDNA sequence in the same reading frame (amino-acid residues 221-233, 304-316 and 354-359). Using this reading frame, the amino-acid sequence of the cryptic portion of the mAChR was deduced (Fig. 2b). The translational initiation site was assigned to the

|| To whom correspondence should be addressed.

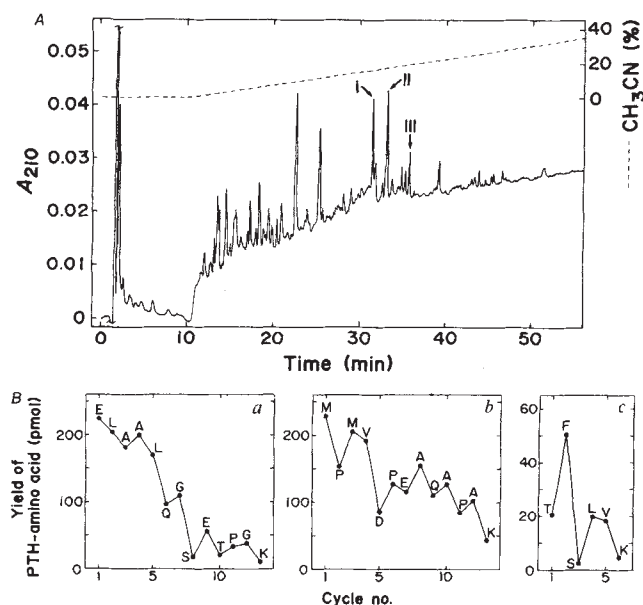


Fig. 1 Partial amino-acid sequence analysis of the mAChR from porcine brain. **A**, Reverse-phase HPLC of tryptic peptides. The tryptic digest was directly loaded onto a Chemcosorb 3μ C₁₈-H column (8×75 mm, Chemco) equilibrated with 0.1% trifluoroacetic acid, and a linear gradient of 0–60% acetonitrile applied for 80 min at a rate of 2 ml min^{-1} ; a Hitachi 635 HPLC system with a solvent programmer was used. The eluate absorbance at 210 nm was recorded with a Hitachi 41 ultraviolet monitor. Fractions corresponding to relatively large absorbance peaks, including fractions I–III, were collected. **B**, Sequence analysis of peptides. The amino-acid sequences determined for fractions I (**a**), II (**b**) and III (**c**), which contained a single peptide, are shown. Sequence analysis was carried out with a gas-phase sequencer¹⁰ (model 470A, Applied Biosystems). Phenylthiohydantoin (PTH)-amino acids were analysed by HPLC at 50°C using an Ultrasphere ODS column (4.6×250 mm, Altex) with a three-solvent gradient (acetonitrile, water and 40 mM sodium acetate buffer, pH 5.6) at a flow rate of 1 ml min^{-1} . The yield of PTH-amino acids at each cycle of Edman degradation is shown; the one-letter amino-acid notation is used.

Methods. The purified mAChR⁷, derived from $\sim 1,350$ g porcine cerebra (obtained from a local abattoir), was dialysed against water and lyophilized. The protein thus obtained (~ 1.1 nmol) was dissolved in $160\mu\text{l}$ 70% (v/v) formic acid and subjected to gel filtration on a Superose 12 column (10×300 mm, Pharmacia) equilibrated with the same solvent to be freed from digitonin/cholate. The protein fraction ($\sim 70\mu\text{g}$), lyophilized and dissolved in $100\mu\text{l}$ 50 mM Tris-HCl buffer, pH 8.0, containing 2 mM CaCl_2 , was digested with $1\mu\text{g}$ TPCK-treated trypsin (Worthington) at 37°C for 36 h; further identical aliquots of TPCK-treated trypsin were added at intervals of 12 h.

methionine codon composed of nucleotide residues 1–3 because this is the first ATG triplet that appears downstream of a non-sense codon, TAG (nucleotides –51 to –49), found in-frame. The sequence surrounding the ATG triplet agrees reasonably well with the favoured sequence that flanks functional initiation codons in eukaryotic mRNAs¹⁴. A translational termination codon (TGA) occurs in-frame following codon 460 specifying cysteine. Thus, we conclude that the porcine cerebral mAChR consists of 460 amino-acid residues (including the initiating methionine), with a calculated M_r of 51,416. The difference between this M_r value and the reported value ($M_r\sim 70,000$)⁷ may be accounted for by the carbohydrate moiety of this glycoprotein¹⁵ and possibly by the inaccuracy inherent in the estimation of M_r of membrane proteins by SDS-polyacrylamide gel electrophoresis¹⁶. Assuming a carbohydrate content of 26.5% by weight, the M_r of the protein moiety of the porcine cardiac mAChR has recently been estimated to be 50,000–60,000 (ref. 17). The amino-acid composition of the mAChR predicted from

the cDNA sequence is generally more similar to that determined for the porcine cerebral mAChR⁷ than to that determined for the porcine cardiac mAChR¹⁷. However, this comparison should be made with reservations because of the inaccuracy associated with the chemical analysis of limiting amounts of glycoprotein.

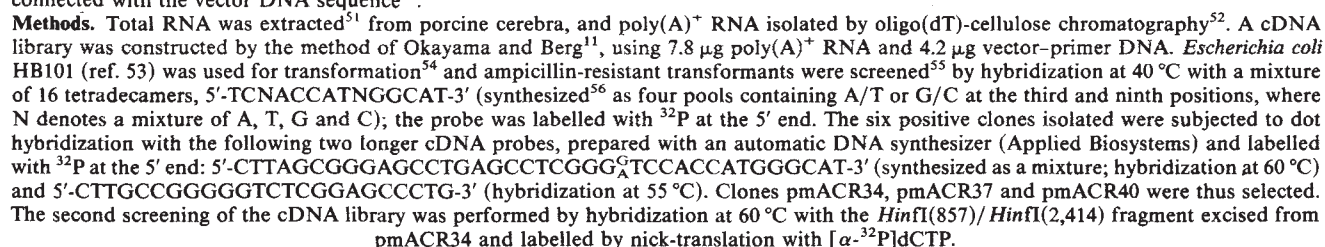
The 3' non-coding region of the cDNA is 1,061 nucleotides long, excluding the poly(dA) tract, and contains the polyadenylation signal¹⁸ AATAAA (nucleotides 2,422–2,427) 15 nucleotides upstream from the poly(dA) tract. The 5' non-coding region of the cDNA contains an open reading frame of 19 codons (or 44 codons) starting with an ATG triplet (residues –108 to –106 or –183 to –181) which is in the same reading frame as the initiation codon; there are additional ATG triplets out-of-frame (residues –325 to –323, –134 to –132 and –95 to –93). The ATG triplets in the 5' non-coding region are flanked by sequences that are less similar to the consensus sequence of Kozak¹⁴ than is the initiation codon. Similarly, an open reading frame of 19 or 20 codons starting with an ATG triplet which is in the same reading frame as the initiation codon has been found at an almost equivalent position in the 5' non-coding region of the hamster lung β -adrenergic receptor (β -AR) cDNA¹⁹ as well as of the human oestrogen receptor cDNA²⁰.

Structural characteristics

Analysis of the amino-acid sequence of the mAChR for local hydropathicity²¹ (Fig. 3) shows the presence of seven hydrophobic segments (I–VII), one of which (VII) is less hydrophobic than the others. This hydropathicity profile is similar to those of bacteriorhodopsin²² and rhodopsin^{23–25} and also to that of the β -adrenergic receptor¹⁹, which has recently been shown to resemble the hydropathicity profiles of the two former proteins. Homology matrix comparison²⁶ reveals that the amino-acid sequence of the mAChR is similar to those of β -AR¹⁹ and rhodopsin²³. Figure 4 shows the alignment of the amino-acid sequences of the three proteins. The degree of sequence identity is 30, 23 and 23% for the mAChR/ β -AR, mAChR/opsin and β -AR/opsin pairs, respectively; a continuous stretch of gaps has been counted as one substitution regardless of its length. In addition, 34 (mAChR/ β -AR), 32 (mAChR/opsin) and 29% (β -AR/opsin) of the amino-acid replacements represent favoured amino-acid substitutions²⁷. Amino-acid sequence homology between β -AR and opsin in the regions corresponding to the hydrophobic segments V, VI and VII has recently been reported¹⁹. These observations suggest that the genes encoding the three proteins arose from a common ancestor.

Electron-diffraction studies indicate that bacteriorhodopsin contains seven α -helical rods spanning the membrane²⁸. This and the primary structure information^{29,30}, together with transfer free-energy calculations, protein modification and other studies²², have suggested a transmembrane topography for the bacteriorhodopsin molecule. A similar transmembrane model for rhodopsin with seven membrane-spanning segments has been derived from its primary structure and other data^{23–25}. The glycosylated amino-terminal region of rhodopsin is located on the intradiskal side of the membrane, whereas the carboxy-terminal region is exposed on the cytoplasmic side. Because the mAChR is similar in hydropathicity profile to these chromoproteins and because its amino-acid sequence is similar to that of rhodopsin, we suggest that the mAChR molecule has a transmembrane topography similar to that of rhodopsin (see Fig. 3).

The seven hydrophobic segments, which represent putative transmembrane α -helices, comprise a continuous stretch of 20–24 uncharged amino-acid residues including many nonpolar residues, except that segments II and III contain an aspartic acid residue (residues 71 and 105). The aspartic acid in segment II is conserved in the β -AR as well as in rhodopsin, whereas that in segment III is shared only by the former (Fig. 4); there is a glutamic acid residue in the interior of segment III of rhodopsin at a position not equivalent to that of the aspartic



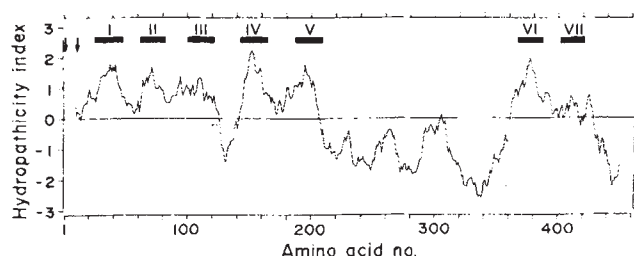


Fig. 3 Hydropathicity profile of the mAChR. The averaged hydropathicity index of a nonadecapeptide composed of amino-acid residues $i-9$ to $i+9$ is plotted against i , where i represents amino-acid number; hydropathicity indices of individual amino acids from ref. 21. Closed boxes, positions of the putative transmembrane segments I-VII; arrows, putative N -glycosylation sites in the amino-terminal region.

acid. The mAChR and the β -AR have no charged counterparts to the histidine in segment V and the lysine in segment VII (the site of retinal attachment^{23,31}) of rhodopsin.

The mAChR does not possess an amino-terminal sequence characteristic of the signal sequence³². The amino-terminal region preceding the putative transmembrane segment I contains two potential N -glycosylation sites³³ (asparagine residues 2 and 12); the two additional potential sites (asparagine residues 110 and 410), which are assigned to the interior of the putative transmembrane segments III and VII, are presumably not glycosylated. These structural features of the amino-terminal region, despite its variance in length, are shared by both the β -AR¹⁹ and rhodopsin^{23-25,31} (see Fig. 4). In fact, the N -glycosylation of rhodopsin at the two potential sites in this region has been demonstrated³¹. Thus, it seems reasonable to assign the amino-terminal region of the mAChR to the extracellular side.

The carboxy-terminal region of the mAChR, which accord-

ingly is assigned to the cytoplasmic side, contains several serine and threonine residues (residues 428, 451, 455 and 457) as potential sites of phosphorylation, as is the case for rhodopsin and β -AR, which are known to be phosphorylated by specific kinases in a light- or agonist-dependent manner^{31,34}; there is evidence for the phosphorylation of clustered serine and threonine residues in the carboxy-terminal region of rhodopsin³¹. The mAChR also contains several potential sites of phosphorylation by a cyclic AMP-dependent protein kinase³⁵, at residues 330, 354, 356 and 451, all of which are assigned to the cytoplasmic side. A large insertion (100-120 amino acids) occurs in the putative cytoplasmic region between the proposed transmembrane segments V and VI of the mAChR compared with the β -AR and rhodopsin (see Fig. 4). The inserted region may be involved in specific functional aspects of the mAChR.

Expression of cDNA

To examine whether the cloned cDNA actually encodes a functional mAChR, we performed expression studies. The cDNA, including the poly(dA) tract, was linked with the bacteriophage SP6 promoter³⁶ and transcribed *in vitro* with SP6 polymerase. The mRNA thus obtained was injected into *Xenopus* oocytes, which were then tested for response to acetylcholine (ACh) under voltage clamp. A typical response of an injected oocyte to ACh applied ionophoretically is shown in Fig. 5A, a. An inward current occurs after a substantial delay (from 1 to 10 s in different oocytes) and is usually oscillatory in nature. The ACh response is abolished after application of 10 nM atropine (Fig. 5A, b), and this effect is reversible (Fig. 5A, c). All the oocytes tested responded to ACh; the peak inward current, measured on oocytes incubated for three days (evoked by bath application of 1 μ M ACh at ~ -70 mV membrane potential), is 1.6 ± 1.1 μ A (mean $\pm$ s.d., $n=40$). In contrast, no detectable response (<10 nA) is observed in 50 control oocytes (noninjected or injected with water); the absence of a measurable ACh response caused by the endogenous mAChR, known to be

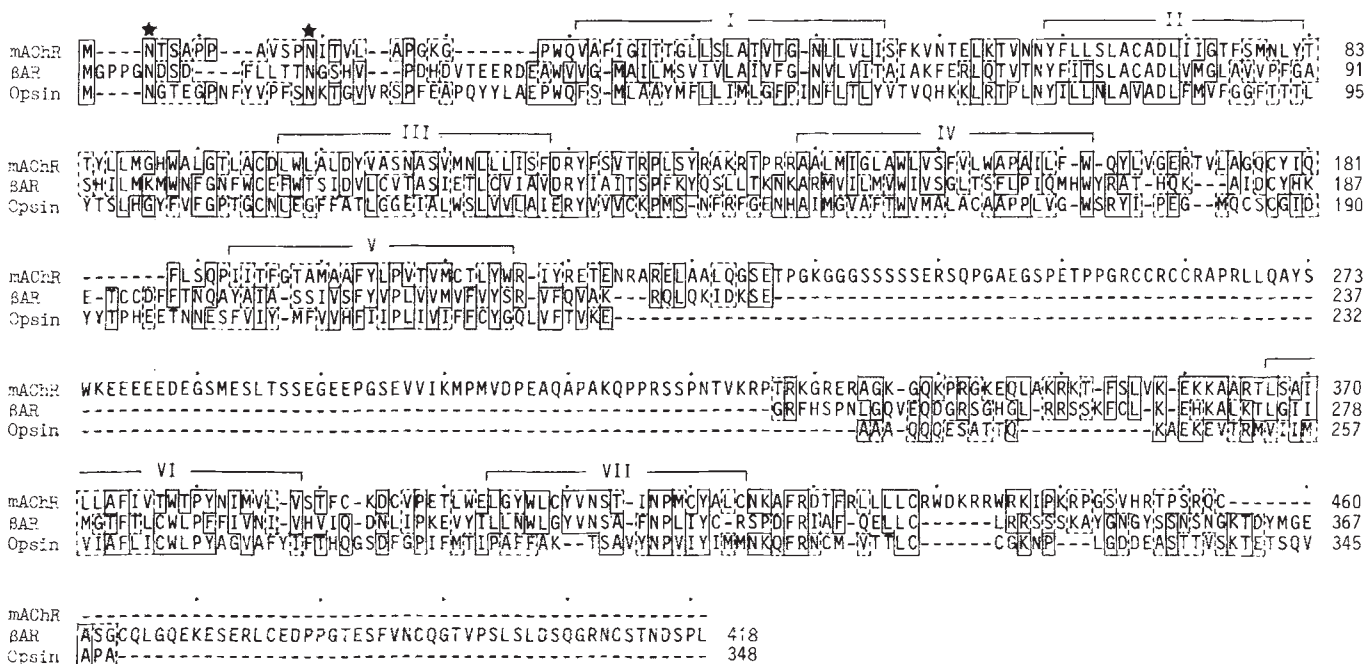


Fig. 4 Alignment of the amino-acid sequences of porcine mAChR (top), hamster β -AR (middle) and bovine opsin (bottom). One-letter amino-acid notation. Sequence data for β -AR and opsin from refs 19 and 23, respectively. Sets of identical residues are enclosed with solid lines and sets of conservative residues with dashed lines. Conservative substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W (ref. 27). Gaps (—) have been inserted to achieve maximum homology. The number of the residue at the right end of each line is given; amino-acid residues are numbered beginning with the initiating methionine. Positions of the putative transmembrane segments I-VII of the mAChR are indicated; the termini of each segment are tentatively assigned on the basis of the hydropathicity profile, the amino-acid sequence and comparison with rhodopsin²³⁻²⁵, but they do not necessarily conform with the termini proposed for rhodopsin in the aligned sequences. Asterisks, asparagine residues as putative N -glycosylation sites in the amino-terminal region.

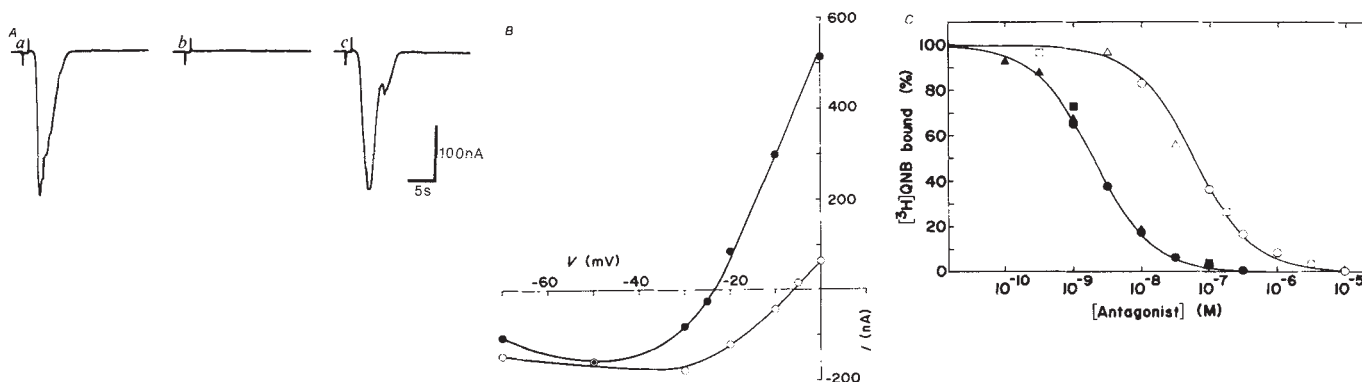


Fig. 5 **A**, ACh responses recorded from a *Xenopus* oocyte injected with the mRNA derived from the cloned mAChR cDNA. Responses to ACh before (**a**) and 1 min after superfusion with 10 nM atropine (**b**) and 6 min after washing out the antagonist (**c**). ACh was applied by constant ionophoretic pulses (intensity, 23 nA; duration, 1 s), and ACh-activated currents (downward deflection indicates inward current) recorded under voltage clamp at -70 mV. **B**, $I-V$ relationship (inward current shown as negative current) observed in Ringer's solution ($\bullet$) and in the solution in which half of the Cl^- was replaced by SO_4^{2-} , the osmolarity adjusted with sucrose ($\circ$). ACh was applied by constant ionophoretic pulses (intensity, 50 nA; duration, 1 s). Peak current activated by ACh is plotted against the membrane potential. **C**, Effects of atropine and pirenzepine on [^3H]QNB binding. Cell extracts prepared from oocytes injected with the mRNA derived from the cloned cDNA were incubated with 0.27 nM [^3H]QNB (specific activity, 42.6 Ci mmol $^{-1}$) in the presence of increasing concentrations of atropine (filled symbols) or pirenzepine (open symbols); different symbols represent measurements with different preparations of cell extracts. Values for 100 and 0% binding were determined by measurements in the absence of atropine or pirenzepine and in the presence of 1 μM atropine, respectively; the 0% values (70–94 d.p.m.) were 4–15% of the 100% values. The theoretical curves have been drawn by nonlinear least-squares analysis according to the equation $y = (1 + x/IC_{50})^{-1}$, where x represents the antagonist concentration. IC_{50} values for atropine and pirenzepine were 2.0 nM and 60 nM, respectively.

Methods. The EcoRI(–171)/BgIII (1,656) fragment and the 890-bp BgIII(1,656)/PvuII (on vector DNA) fragment, derived from clone pmACR84, and the 3,033-bp SmaI/EcoRI fragment from the plasmid pSP65 (ref. 36) were ligated to yield the plasmid pSPM10. The mRNA specific for the porcine mAChR was synthesized *in vitro*³⁶ using XbaI-cleaved pSPM10 as the template. Transcription was primed⁵⁷ with the cap analogue diguanosine triphosphate (0.5 mM). The mRNA was injected into *Xenopus laevis* oocytes (mRNA concentration $0.2 \mu\text{g} \mu\text{l}^{-1}$ for electrophysiological measurements or $0.4 \mu\text{g} \mu\text{l}^{-1}$ for ligand binding assays; average volume injected per oocyte, ~ 40 nl). The injected oocytes were incubated at 19°C for 3–5 days in modified Barth's medium⁵⁸ containing cefmenoxime (0.1 mg ml $^{-1}$) and mycostatin (15 $\mu\text{g} \text{ml}^{-1}$). All electrophysiological measurements were performed at 20 – 22°C in Ringer's solution of the following composition (in mM) unless otherwise specified: NaCl, 115; KCl, 2.5; CaCl_2 , 1.8; HEPES, pH 7.2, 10. After removal of the follicular cell layer⁵⁹, oocytes were held in a 0.2-ml recording chamber through which Ringer's solution was superfused continuously at a rate of 2.5 ml min^{-1} . ACh responses were recorded with a conventional two-micropipette voltage clamp. The two pipettes were filled with 3 M potassium acetate and ACh applied either ionophoretically through a pipette filled with 1 M ACh (backing current 13 nA) or to the bath by superfusing with Ringer's solution containing ACh for 25 s. For ligand binding assays, cell extracts were prepared at 4°C as follows. Oocytes (20–40) were homogenized in 1 ml 10 mM potassium phosphate buffer, pH, 7.0, containing 1 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride, $1 \mu\text{g} \text{ml}^{-1}$ pepstatin and 0.32 M sucrose. The homogenate was centrifuged at 1,000g for 10 min and the precipitate suspended in 1 ml of the same buffer, homogenized and centrifuged at 1,000g for 10 min. The two supernatants were combined and used for binding assays. [^3H]QNB binding activity was determined as described previously⁶⁰, except that incubation was carried out for 1 h; the amount of [^3H]QNB binding in the presence of 1 μM atropine was subtracted to eliminate nonspecific binding.

present in *Xenopus* oocytes^{37,38}, may result from seasonal or individual variations^{38,39}.

The nature of the ACh response observed in oocytes injected with the mRNA resembles that reported for the native mAChR in *Xenopus* oocytes³⁷ and contrasts with the fast non-oscillatory ACh response elicited in oocytes injected with mRNAs specific for the nicotinic acetylcholine receptor subunits⁴⁰ or with poly(A)⁺ RNA from *Torpedo* electric organ⁴¹. Carbamylcholine, muscarine, pilocarpine and arecoline (1 μM each) also induce inward currents in oocytes injected with the mRNA derived from the cloned mAChR cDNA. Scopolamine (40 nM), quinuclidinyl benzylate (QNB) (10 nM) and pirenzepine (0.5 μM) abolish the ACh response in a reversible manner, whereas (+)tubocurarine (2.5 μM) and α -bungarotoxin (0.1 μM) have no effect.

Figure 5B shows the current-voltage ($I-V$) relation for the ACh response. The mean reversal potential of the ACh-activated current, obtained in Ringer's solution (filled circles), is -25.6 ± 2.2 mV ($n = 11$), close to the equilibrium potential of chloride ions observed in *Xenopus* oocytes^{37,38,42}. In fact, the reversal potential shifts by 17.9 ± 1.6 mV ($n = 6$) to a more positive value when the external Cl^- concentration is halved by substitution with SO_4^{2-} (Fig. 5B, open circles), but it does not significantly change when the external Na^+ concentration is decreased threefold by substitution with Tris or when the external K^+ concentration is increased threefold by addition of K_2SO_4 . Thus, the ACh response is principally caused by an increase in the Cl^- conductance of the oocyte membrane, as is the case for the native oocyte mAChR^{37,38}. These results indicate that the mRNA generated by transcription of the cloned cDNA directs the formation of functional mAChR.

The mAChR produced in oocytes was also examined for ligand binding properties. Extracts prepared from oocytes injected with the mRNA exhibit [^3H]QNB binding activity ranging

from 3.2 to 6.8 fmol per oocyte, whereas no measurable activity (<0.1 fmol per oocyte) is observed with extracts derived from control oocytes (noninjected or injected with water). The apparent dissociation constant (K_D) for [^3H]QNB was estimated by Scatchard analysis to be 64 pM, and those for atropine and pirenzepine, obtained by measuring displacement of [^3H]QNB binding (Fig. 5C), are 0.38 nM and 12 nM, respectively, in general agreement with the reported values^{4,6,43,44}. Thus, the mAChR expressed from the cloned cDNA exhibits relatively high apparent binding affinity for pirenzepine. The observed K_D value for this antagonist compares fairly well with that of the crude solubilized preparation (45 nM) but is lower than that of the purified preparation of porcine cerebral mAChR (286 nM)⁷. The interpretation of measured affinities for pirenzepine is complicated by the fact that they are influenced by environmental factors (see, for example, ref. 45). Our ligand binding assays were carried out in the absence of a detergent, although the environmental constraint imposed by the oocyte membrane is not known.

Tissue location of mRNA

Because of the different tissue locations of the mAChR subtypes^{4–6}, we examined several porcine tissues for the content of the RNA hybridizing with the cloned mAChR cDNA. Poly(A)⁺ RNA from the cerebral cortex, corpus striatum, medulla-pons and heart was subjected to blot hybridization analysis with a cDNA probe containing the entire protein-coding sequence (Fig. 6). The cerebral cortex (lane 1) and corpus striatum (lane 2), known to be representative sites for the M₁ subtype, contain an RNA species of $\sim 3,000$ nucleotides that hybridizes with the probe; this size is only slightly larger than that of the cloned cDNA (2,884 nucleotides) if the poly(A) tail is taken into consideration. The difference in the relative contents of the hybridizable RNA in the cerebral cortex and corpus striatum is

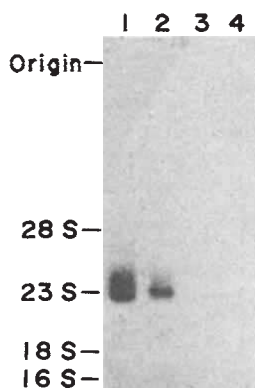


Fig. 6 Autoradiogram of blot hybridization analysis of poly(A)⁺ RNA from different porcine tissues with an mAChR cDNA probe. Each brain tissue used was a pool from 15 individuals, and atria from five individuals were pooled. Poly(A)⁺ RNA (15 µg) from cerebral cortex (lane 1), corpus striatum (lane 2), medulla-pons (lane 3) and atrium (lane 4) was denatured with 1 M glyoxal and 50% dimethyl sulphoxide⁶¹, electrophoresed on a 1.5% agarose gel and transferred⁶² to a Biodyne nylon membrane (Pall). The hybridization probe used (specific activity 8×10^7 c.p.m. µg⁻¹) was an equimolar mixture of the *Eco*RI(-171)/*Hinf*I(857) fragment and the *Hinf*I(857)/*Hinf*I(2,414) fragment excised from clone pmACR84 and labelled by nick-translation with [α -³²P]dCTP. The hybridization and washing conditions were the same as described previously⁶³, except that the concentration of the cDNA probe was 5 ng ml⁻¹. Autoradiography was at -70 °C for 60 h with intensifying screen. Size markers were porcine and *E. coli* rRNAs⁶⁴.

not so evident in other experiments. In contrast, no hybridizable RNA is detected with the preparations from the medulla-pons (lane 3) and heart (lane 4), which are known to contain preferentially the M₂ subtype; under the conditions used here, corpus striatum poly(A)⁺ RNA yields a detectable hybridization signal even when its amount is reduced 10-fold. These results are consistent with the notion that the cDNA we have cloned is derived from the mRNA encoding the mAChR of the M₁ subtype. In accord with this notion is the high apparent binding affinity for pirenzepine observed for the mAChR expressed from the cDNA. Thus, it is tempting to assume that the mAChRs of

the M₁ and M₂ subtypes are encoded by different mRNA species. Attempts to link the mAChR subtypes based on affinity for pirenzepine with specific muscarinic responses such as adenylate cyclase inhibition and phosphoinositide breakdown have so far been unsuccessful^{45,46}.

Discussion

Cloning and sequence analysis of the cDNA reveal that the mAChR resembles the β -AR and rhodopsin in amino-acid sequence as well as in hydropathicity profile, suggesting that the three proteins evolved from a common ancestor. The three proteins have several structural characteristics in common. First, they contain seven putative transmembrane segments. Second, their amino-terminal region is devoid of the signal sequence³² and contains two putative N-glycosylation sites; these sites in rhodopsin are known to be glycosylated³¹. Third, their carboxy-terminal region has several serine and threonine residues as putative sites of phosphorylation; the phosphorylation of these residues in rhodopsin has been demonstrated³¹. In addition, the three proteins share common functional features in that they are cell surface receptors that interact with G proteins to transduce signals into cellular responses^{3,8,9,47,48}. It has recently been shown⁴⁹ that the three cone pigments share structural characteristics with rhodopsin; thus these visual pigments represent a family of related gene products. There are pharmacologically distinguishable subtypes of the mAChR as well as of the β -AR, and our results favour the possibility that the subtypes of the mAChR represent different gene products. Furthermore, the putative mating factor receptors of yeast⁵⁰ possess seven putative transmembrane segments and share many of the structural features with the mammalian cell-surface receptors discussed here. Thus, it is tempting to assume that all these proteins which seem to have seven membrane-spanning regions are evolutionarily related and represent a family of widely distributed cell-surface receptors that are linked to G proteins.

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An emission-line object in the core of M15

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Optical identification of globular cluster X-ray sources is essential if their evolutionary state and relation to the galactic bulge sources is to be understood. The 10 high-luminosity ($\geq 10^{36}$ erg s $^{-1}$) X-ray sources associated with globular clusters have very accurate (~ 1 arc s) positions from the Einstein Observatory survey^{1,2}. No globular cluster source has yet been optically identified, primarily because all of the bright X-ray sources are located close to the dynamical centre (implying total masses of $\sim 1.5M_{\odot}$ (ref. 2)), where crowding makes optical photometry and spectroscopy extremely difficult. The only northern cluster containing a bright X-ray source is M15, which has the extremely ultraviolet ($U-B = -1.2$) and variable star AC211 near the X-ray error circle^{3,4}. The reported spectrum is that of an A star^{5,6}, with no emission lines which are normally present in galactic low-mass X-ray binaries. We have now discovered^{7,8} strong He II 4,686-Å emission from the region of AC211, and weaker Balmer emission. We conclude that it is an intrinsically high-luminosity ($L_X \geq 10^{38}$ erg s $^{-1}$) X-ray source on the basis of the optical brightness of the star and the He II flux. As the observed L_X is only 6×10^{36} erg s $^{-1}$, this implies that the system is at a high inclination and that only scattered X-rays from an extended region are observed. The mass transfer rate required to account for this is very high and places important constraints on the evolutionary state of the mass-losing star which, for its optical brightness, must either be a red giant or horizontal branch star. The location of AC211 questions the accuracy of the Einstein Observatory positions.

The core of M15 (Fig. 1) was observed with the Isaac Newton Telescope of the Observatorio del Roque de los Muchachos on 26 June 1985 between 04:00 and 05:00 UT. The RGO (Royal Greenwich Observatory) intermediate dispersion spectrograph was used with the 235-mm camera and IPCS (image photon counting system) which, together with a 300 line mm $^{-1}$ grating,

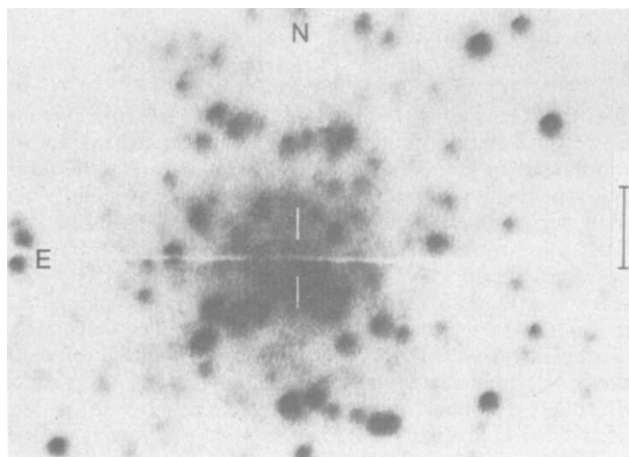


Fig. 1 The core of M15 as seen through the Isaac Newton Telescope Acquisition and Guidance system, digitized and displayed on the Oxford image processing system. The 0.5 arc s E-W entrance slit of the spectrograph is clearly visible and the vertical bars indicate the location of the AC207/211 region. A detailed finding chart for the central 10×10 arc s region can be found in ref. 3, although note that the orientation is significantly different from that shown here. Scale bar, 10 arc s.

yielded a dispersion of 138 Å mm $^{-1}$ (resolution ~ 6 Å) and covered the range 3,700–7,800 Å (calibrated with a Cu–Ar arc). However, the IPCS is a two-dimensional detector and spatially resolved spectra along the slit were obtained with a resolution of 1.5 arc s covering most of the east–west width of fig. 1. Seeing conditions were ~ 1.2 arc s.

The 0.5 arc s slit width was used to minimize crowding effects, but this can yield additional problems due to atmospheric dispersion and indeed the parallactic angle was only 23°. However, the source was observed very close to the meridian (zenith distance = 18°) and the effects of atmospheric dispersion were computed to be much smaller than the slit width. Four 500-s exposures were made with only slight north–south adjustments between them, with the aim of maximizing the signal from the obviously ultraviolet (even in the real-time display) object. The counts in each spectrum along the slit for the sum of the middle two exposures are shown in Fig. 2, which reveals the presence of an ultraviolet object.

The ultraviolet flux peaks in spectrum 29 and we show in Fig. 3 spectra 28–31. We have not attempted to subtract any 'background' spectrum for reasons that are obvious from Fig. 2. The ultraviolet nature of spectrum 29 is remarkably clear, but even more dramatic is the strong He II 4,686-Å and weaker H α emission which appears in the adjacent spectrum 30. The apparent separation of the ultraviolet continuum and the emission line is attributed to the difficulty, at this resolution, of separating stars AC207 and AC211 (refs 7, 8). AC207 is itself ultraviolet³ and is always the brighter star in the B and V bands, with AC211 only matching it in the U band at maximum brightness. The similarity of spectrum 29, and that of ref. 5 should be noted. There is also evidence in our four separate spectra (Fig. 4) for significant variations in the He II profile.

The discovery of He II emission (indicating the presence of X-ray-heated gas) means that we have confirmed the identification of AC211 with the M15 X-ray source. However, this result has considerable consequences for the accuracy of the X-ray locations from the Einstein survey². The quoted 0.6 arc s radius (1σ) circle is very small due to multiple HRI (High-resolution imager) pointings. Unfortunately, AC211 is 2.6 arc s from the HRI location and hence outside the 90% confidence region.

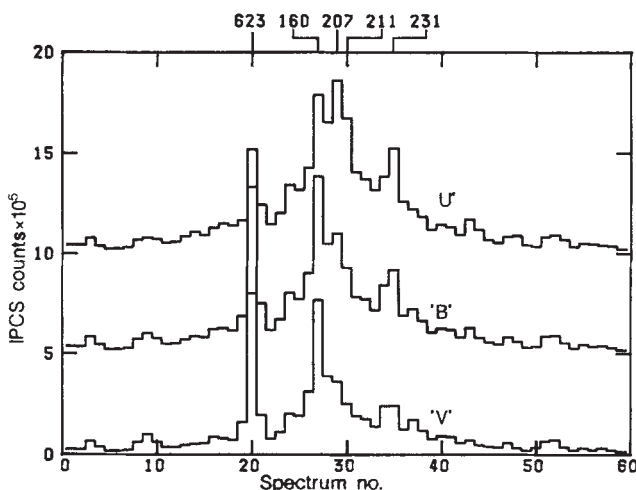


Fig. 2 A one-dimensional cross-section of M15 (along the E–W slit of Fig. 1) in three 'colours', obtained by integrating the spectra in the 'U' (3,700–4,200 Å), B (4,000–5,200 Å) and V (5,000–6,600 Å) pass-bands. Note that our 'U' represents only about half of the normal wavelength range. The star numbers are from ref. 3. The clear ultraviolet peak is due to the blend of AC207 and 211 which caused the initial confusion^{7,8}. Note that each cross-section is offset from the one below by 5×10^5 counts and that the 'U' cross-section has been multiplied by a factor of 5.

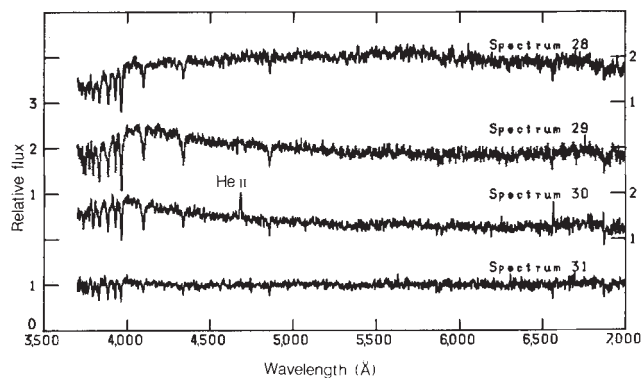


Fig. 3 The four IPCS spectra around the AC211 region. To show the relative continuum shapes, all the spectra are divided by a fifth-order polynomial fit to spectrum 31. We interpret spectrum 29 as AC207 and 30 as AC211. Note that these two spectra are substantially more ultraviolet than the others. The flux axis is labelled on the left for spectra 29 and 31, and on the right for 28 and 30.

The mean wavelength of the He II line from the sum of the four integrations is $4,681.8 \pm 0.3 \text{ \AA}$ and the full-width at half maximum (FWHM) is $10.0 \pm 0.7 \text{ \AA}$. The uncertainties are obtained from gaussian fits to the line profiles. The heliocentric systemic velocity is $-230 \pm 18 \text{ km s}^{-1}$, significantly larger than the cluster's overall velocity⁹ of -100 km s^{-1} . The mean absorption line velocity for the core region is also significantly shifted from the cluster velocity as had been noted earlier⁶, but this was not confirmed by higher-resolution observations in November 1985.

The intensity of the He II line is much harder to measure due to the obvious crowding effects which leave the emission-line object's continuum contaminated from stars on all sides. This means that our measured equivalent width of $3 \text{ \AA}$ must be treated as a lower limit.

The spatial resolution and slit width of the observations reported here make it impossible to estimate accurately the relative intensities of the stars along the slit. Nevertheless, we can use the existing CCD (charge-coupled device) photometry^{4,10} as a guide in discussing the optical and X-ray properties of the M15 X-ray source. These and other parameters are summarized in Table 1.

The relevant properties of this system are: (1) the very large modulation in U with correspondingly small changes in B. This implies X-ray heating of the face of the companion star, coupled with a high inclination^{3,6}; (2) the ratio of X-ray¹¹ to optical fluxes is at least a factor of 10 below that of the 'classical' galactic bulge X-ray sources, but is comparable with that of 'accretion disk corona' sources such as 2S0921-630, 2A1822-371 and 4U2129+47^{3,12,13}. In these latter sources, we do not view the X-ray source directly, but only a small fraction of the

Table 1 X-ray and optical data on M15/AC211

Band	Bright	Faint	Ref.
U	14.6	15.9	10
B	15.8	16.3	10
V	15.9	16.4	10
X-ray flux (μJy)	3-50*		11
Distance (kpc)	7.1		5
L_X (erg s^{-1})	6×10^{36}		1
$(m-M)_V$	14.26		5
$E(B-V)$	0.08		3

* This represents a long-term variation whose relation to the optically bright and faint states of AC211 is unclear.

Table 2 Two models for the M15 X-ray source

	(1) Long-period red-giant system	(2) Short-period horizontal-branch system
$M_c (M_\odot)$	0.8*	0.6†
$R_c (R_\odot)$	8.2	0.67
P	9.7 days	6.2 h
$q (M_x/M_c)$	2	2.5
$a (R_\odot)$	26	2.2
R_c/a	0.32	0.30
$L_X^i (\text{erg s}^{-1})$	1.5×10^{38}	1.9×10^{38}

* Such a red giant requires¹⁴ a core mass of $0.23 M_\odot$.

† We also adopt $\log T_{\text{eff}} = 4.1$ (refs 18, 19) for the M15 horizontal branch²⁰.

X-rays through scattering in a corona or wind above the disk. The necessarily high inclination produces optical and X-ray eclipses, sometimes partial.

Unfortunately, the three systems mentioned above for comparison with AC211 have periods from 5 hours to 9 days and hence have mass-losing components ranging from late-type main-sequence to giant stars. We do not (yet) know the binary period of AC211, but, unusually for galactic X-ray sources, we do know the distance fairly accurately⁵ and can therefore proceed as follows.

Adopting an unreddened B_0 of 15.5 and the same bolometric correction as for Sco X-1 and MXB1735-44¹², we find that the bolometric flux of AC211 is $F_{\text{bol}} = 1.1 \times 10^{-10} \text{ erg cm}^{-2} \text{ s}^{-1}$ and hence the peak $F_X/F_{\text{bol}} = 7.5$ (or $B_0 - m_X = 19.75$)¹². This corresponds to a bolometric luminosity L_{bol} of $6.3 \times 10^{35} \text{ erg s}^{-1}$ at the distance of M15. Assuming that mass transfer is by Roche lobe overflow, then we have the size R_c of the companion given by the standard relation

$$R_c = 0.234 P^{2/3} M_c^{1/3} R_\odot$$

where the period P is in hours and the companion mass M_c is in $M_\odot$. ($M_\odot$ and $R_\odot$ are the mass and radius of the Sun.) We will also assume that the dominant ultraviolet component is due to X-ray heating and hence that

$$L_{\text{bol}} = 1/4 (R_c/a)^2 L_X^i (1 - \alpha)$$

where a is the binary separation, α is the X-ray albedo and L_X^i is the intrinsic X-ray luminosity. Taking $\alpha = 0.5$ then

$$L_X^i = 8 L_{\text{bol}} (R_c/a)^{-2}$$

From these equations, we examine two representative models, one of short period, the other long, assuming masses for M_c appropriate for globular cluster members and using the faint-state unreddened visual luminosity of $16 L_\odot$ as a guide to the parameters of the companion star. M_X is always taken as a $1.5 M_\odot$ neutron star. The results are summarized in Table 2.

Both calculations yield a very high X-ray luminosity (much greater than that observed directly), close to the Eddington limit for our $1.5 M_\odot$ neutron star, and hence need very high mass transfer rates $\sim 10^{-8} M_\odot \text{ yr}^{-1}$. Evolutionary models of close binaries¹⁴ cannot sustain these high rates from low-mass main-sequence companions, but they are possible in red-giant systems. It has been suggested⁶ that one very high-luminosity system would be expected amongst the X-ray globular cluster sources. In fact, M15 is the only X-ray globular cluster not producing bursts, as expected if the mass transfer rate is high. However, models of mass transfer from horizontal-branch stars in binaries have not yet been investigated, although it has been suggested¹⁵ that high rates can occur.

Our discovery of the He II emission line provides additional support for a high L_X^i . Assuming an underlying $V = 15$ continuum for our spectrum 30, then the He II flux is at least

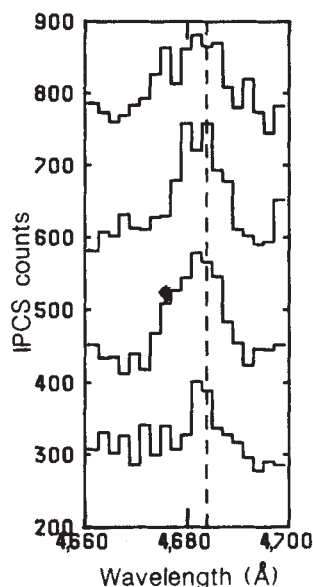


Fig. 4 The four separate 500-s integrations (first at the top), showing the He II profile of spectrum 30. Variations in the profile width are evident. The dashed line marks the heliocentric systemic velocity of M15 (see text). The width of the last profile is consistent with the instrumental resolution of 5.3 Å FWHM (determined from arc spectra). The zero of each plot is offset by 100 counts from the one below.

$1.3 \times 10^{-14} \text{ erg cm}^{-2} \text{ s}^{-1}$, or $8 \times 10^{31} \text{ erg s}^{-1}$ at M15. This is at least 10 times higher than that of Sco X-1¹⁶. If we assume that the He II is directly related to the level of X-ray emission then we may use the observed ratio of L_X/L_{4686} in Sco X-1 ($\sim 3 \times 10^6$) to infer an L_X of $3 \times 10^{38} \text{ erg s}^{-1}$ for M15, a factor of 50 greater than that actually observed (Table 1). The fact that large optical variations^{3,4,10,17} have been seen frequently may argue against a long-period system, whereas more detailed theoretical models are required for the short-period case. However, only further observations can define the basic model of M15, and, if the He II arises on the heated face of the secondary, then a radial velocity study will be of enormous value. As the only known optical counterpart of a bright globular cluster X-ray source, it will surely be studied intensively.

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Particle acceleration in the hotspot of the jet of quasar 3C273

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Recent radio, optical and near-infrared observations of the dominant hotspot at the outer end of the quasar jet 3C273A have revealed a complex spectrum, significantly different from a simple power law, which provides a unique probe of the acceleration process supplying the synchrotron-emitting electrons. Here we present theoretical calculations of the electron spectrum produced by non-relativistic shock acceleration which, for the first time, include both synchrotron losses and a finite emission region. The resultant synchrotron spectrum fits the observations, providing a natural explanation for the steep spectrum at radio to infrared frequencies, the observed flattening at $\nu < \nu_b = 1.5 \text{ GHz}$, and the cutoff above $\nu_c = 2 \times 10^{14} \text{ Hz}$. Our model yields a new magnetic field estimate of 70 nT, remarkably similar to that derived from minimum-energy calculations, as well as an estimate for the electron mean free path. We predict that the optical emission region is only $\sim 1 \text{ pc}$ thick, increasing with decreasing observation frequency as $\nu^{-1/2}$.

The jet of the nearby quasar 3C273 terminates at 21.4 arc s from the core in a bright radio hotspot which contributes 80% of the total jet flux at 5 GHz (refs 1-5). Multi-colour charge-coupled device (CCD) observations⁶ and near-infrared photometry⁷ revealed that the absence of a similar optical hotspot is due to a sharp cutoff in the hotspot spectrum above $\sim 2 \times 10^{14} \text{ Hz}$. On high-resolution radio maps³ the hotspot region is resolved into three well-separated subcomponents (1, 2 and 3 in ref. 3). The brightest (component 2) is highly polarized ($P > 20\%$) and contributes half the total flux. On a 15-GHz VLA (Very Large Array) map (0.22 arc s resolution; R. A. Perley, personal communication) this subcomponent is elongated perpendicular to the jet axis (minor: major axis $< 2:3$). The magnetic field is oriented perpendicular to the jet axis in component 2, whereas it is aligned with the jet everywhere else. The field orientation and high synchrotron brightness point to component 2 being the strong planar shock seen in hydrodynamic simulations of jet flows^{8,9}, and therefore the most likely place for the required *in situ* acceleration of relativistic electrons. This is supported by unpublished VLA spectral index maps (R. A. Perley, personal communication), which show a steepening of the spectral index with distance outward from component 2.

We consider here whether Fermi acceleration at a strong shock in component 2 can account for the overall hotspot spectrum. As the angular resolution of ground-based optical telescopes is insufficient to resolve the hotspot into its subcomponents, our discussion of the radio to optical spectrum must refer to the entire hotspot region beyond 20 arc s from the quasar core. The total flux (S_ν) values are given in Table 1. For the first time, the spectrum of a bright radio hotspot is well constrained both at low and high frequencies (ν) (see Fig. 1). Even assuming a

Table 1 Total hotspot flux values between 400 MHz and 5×10^{14} Hz

Frequency (Hz)	Flux, S_ν (Jy)	Two-point spectral index $-d(\ln S_\nu)/d(\ln \nu)$	FWHM of component 2 [arc s]	Beam size [arc s]	$S(2)/S(\text{total})$
0.4×10^9	30.5*		Unresolved	0.9	
1.67×10^9	11.2*	0.72	0.60×0.67	0.35	0.44
4.9×10^9	4.3†	0.90	0.66×0.71	0.35	0.50
15×10^9	1.43*	1.00	0.44×0.67	0.22	0.42
1.36×10^{14}	$100 \pm 20 \times 10^{-6} \ddagger$	1.05	Unresolved	8.0	
2.40×10^{14}	$50 \pm 10 \times 10^{-6} \ddagger$		Unresolved	8.0	
3.47×10^{14}	$7.8 \pm 1.1 \times 10^{-6} \S$	5.2	Unresolved	1.7	
4.89×10^{14}	$1.3 \pm 0.5 \times 10^{-6} \S$		Unresolved	1.5	

* Based on the total flux at 4.9 GHz and the spectral indices (from ref. 4 and unpublished VLA data; R. A. Perley, personal communication).

† Integrated flux beyond 20 arc s from the quasar core (VLA map).

‡ We have assumed a simple power law, $S_\nu \propto \nu^{-0.96}$, for the contribution of the 'optical knot' in the 8 arc s infrared apertures (see ref. 6).

§ From ref. 6.

smooth spectrum between the radio and the infrared, it is clear that the spectrum does not follow the simple power law, $S_\nu \propto \nu^{-0.5}$, predicted by standard shock acceleration theory¹⁰. The simplest electron energy distribution required to account for the emission is $N(\gamma) \propto \gamma^{-2}$ (where γ is the electron energy/ $m_e c^2$) at low energies (as predicted by the standard theory), with a break at γ_b to a steep γ^{-3} and a cutoff at γ_c (dotted line in Fig. 1).

We show here that first-order Fermi acceleration can indeed produce the desired electron spectrum, provided account is taken of both synchrotron losses and the finite extent of the emission region. Our assumptions are: (1) the jet speed $u_j = \beta_j c$ is non-relativistic at the shock; (2) the shock is strong (compression ratio $r = 4$) and planar; (3) the synchrotron-emitting electrons are contained in a region where the flow pattern is essentially one-dimensional; (4) the scattering mean free path λ_{ei} for the relativistic electrons is independent of energy, and small compared with the physical scales in the region; (5) the mechanism has reached a steady-state.

Figure 2 shows the calculated electron distribution at different distances $x = (4\lambda_{ei}/3\beta_j)Z$ downstream of the shock. At the shock, the balance of Fermi acceleration and synchrotron losses results in a quasi-exponential cutoff at $\gamma = \gamma_c$ (refs 11–13). At low energies, $\gamma \ll \gamma_c$, synchrotron losses are not important and the spectrum is the expected power law, $N(\gamma) \propto \gamma^{-2}$. Downstream, the cutoff moves to lower energies, due to synchrotron losses and the fact that only electrons with $Z \leq 1$ are able to re-cross the shock and gain more energy¹⁴. If the emission region is of finite extent (either fixed by telescope beam, or by a physical boundary), the integrated spectrum $\int_0^{\text{max}} N(\gamma, Z) dZ$ has the required properties, with a break to γ^{-3} at γ_b (Fig. 2). The problem is similar to that of synchrotron ageing of an injected power-law spectrum (see ref. 15). The observed break frequency delineates those electrons (with $\gamma > \gamma_b$) that lose a significant fraction of their energy in crossing the emission region (length $L = L_{\text{kpc}}$ kpc downstream of the shock) from those that do not. As can be seen from Fig. 1, the theoretical spectrum agrees well with the observed data, although the high-energy cutoff is smoother than observed (however, there is some uncertainty in separating the contribution from the inner parts of the jet). Note that, apart from normalization, the model has essentially only one free parameter—the ratio between the cutoff frequency and the break frequency:

$$(\nu_c/\nu_b)^{1/2} \approx \gamma_c/\gamma_b = 1 + Z_{\text{max}}/5 \quad (1)$$

with $Z_{\text{max}} = 3L\beta_j/(4\lambda_{ei})$. Best fits to the observed spectrum give $\gamma_c/\gamma_b = 320 \pm 30$ and $\nu_b = 42$ Hz ($B/1$ nT) $\gamma_b^2 = 1.5 \pm 0.6$ GHz.

We have assumed that the electrons diffuse, and are therefore convected with the fluid. This allows us to estimate the magnetic field B , from the condition that, for the electrons emitting near the break frequency, the synchrotron loss-time equals the time

for the fluid to be convected through the emission region. This yields

$$B = 250 \text{ nT } \beta_j^{2/3} L_{\text{kpc}}^{-2/3} (\nu_b/1 \text{ GHz})^{-1/3} \quad (2)$$

Figure 3 shows B as a function of β_j . The parameters γ_c/γ_b , β_j and L also determine the electron scattering mean free path:

$$\lambda_{ei} = 0.75 L (\gamma_b/\gamma_c) \beta_h \quad (3)$$

Further details will be given elsewhere²².

The underlying assumptions either have a sound physical basis, or can be relaxed without changing the essential results. Assumptions (2) and (3) are supported by hydrodynamic models of jet flows⁹. In such models, there is a region downstream of the Mach disk where the flow is essentially one-dimensional, before pressure effects cause the flow to diverge. Indeed, the physical boundary of the emission region may be set by the place where the flow diverges and both the electron density and the magnetic field decrease. Assumption (4) is made for mathematical tractability. However, the only result which depends on the value of the mean free path is the cutoff energy. If the scattering is produced by self-generated Alfvén waves, the mean free path is expected to increase with energy¹⁰, improving the fit by causing a sharper cutoff. The estimate in equation (3) would then apply to the electrons with $\gamma \approx \gamma_c$. The magnetic field estimate and the form of the spectrum require only that $\lambda_{ei} \ll L$. Relativistic shock acceleration is rather poorly understood, so assumption (1) must be made. However, provided that shock acceleration takes place in a thin layer ($\ll L$), producing a power law $N(\gamma) \propto \gamma^{-2}$ up to a cutoff, and the electrons diffuse rather than stream freely, the present results will still hold. In the model, we can estimate the acceleration timescale (~ 10 yr to increase γ by a factor e), to justify *a posteriori* our assumption (5).

Evaluation of equations (2) and (3) requires an estimate of the length L of the downstream region. The main uncertainty is the angle, θ_j , between the jet and the line of sight. For any field geometry which is symmetrical with respect to the jet axis, the observed linear polarization $P = 25\%$ (ref. 3) implies $\theta_j > 47^\circ$ (ref. 16). If one interprets the apparent shape of component 2 (at 15 GHz) in terms of an inclined circular disk, one finds again $\theta_j \approx 45^\circ$.

This limit is at variance with the orientation of the superluminal expansion observed within 5 arc ms of the quasar core ($\theta_{\text{mas}} \leq 10^\circ$; see ref. 17). The discrepancy could be explained in several ways. First, Flatters and Conway³ have put forward the hypothesis that the outermost hotspot component 3 represents the strong shock, while component 2 is a 'knot' in the jet flow, enhanced in brightness by relativistic beaming. An aberration effect would then account for the high apparent polarization. It is hard to see how the magnetic field flip and the relatively flat

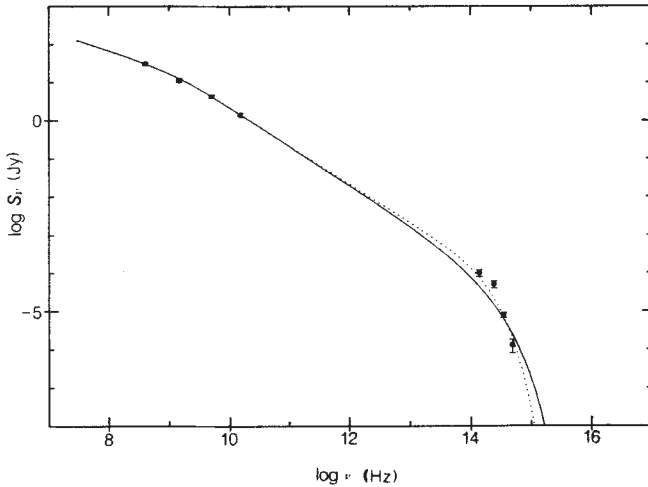


Fig. 1 Radio to optical spectrum of the hotspot in the jet of 3C273. The observed data (see Table 1) are fitted by the synchrotron spectrum inferred from the Fermi acceleration model. The dotted line shows the synchrotron spectrum of the simplified electron spectrum (see text), with an instantaneous cutoff at $\gamma = \gamma_c$. Both synchrotron spectra are calculated by assuming a magnetic field which is randomly orientated in a plane, but we have found that the spectral shape is insensitive to variations of the field geometry.

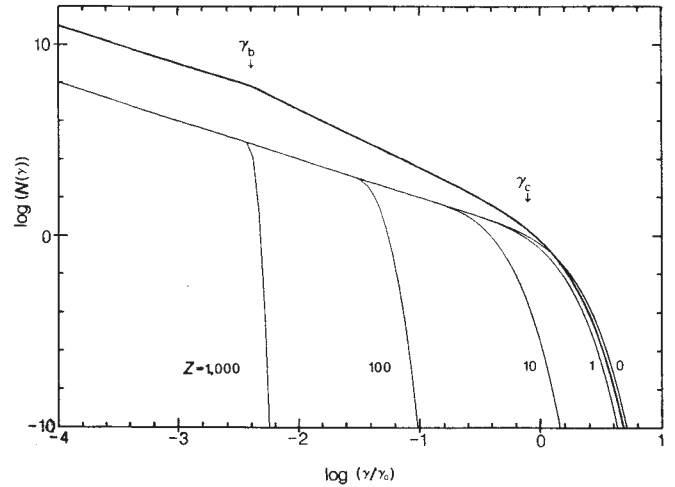


Fig. 2 Energy spectrum of the electrons at different normalized distances Z downstream from the shock. The units of Z are $4\lambda_{ei}/3\beta_j$, where $c\beta_j$ is the jet speed relative to the shock and λ_{ei} is the mean free path of the electrons. The thick line shows the spectrum integrated over a finite size ($0 < Z < 1,000$) of the emission region. The integrated spectrum is proportional to γ^{-2} at low energies, has a break to γ^{-3} at $\gamma = \gamma_b$, and a quasi-exponential cutoff near γ_c .

spectral index of component 2 arise in their model, whereas identifying component 2 with the strong shock provides a natural explanation for these properties. Second, the field and shape of component 2 may be intrinsically asymmetric and the apparent perpendicular orientation arises by chance. In this case $\theta_j \approx 10^\circ$ may well be possible and the length L is poorly constrained by the appearance of the hotspot. We would then have to base an estimate of L on the hydrodynamical models alone (see below). Third, there may be an actual misalignment between the direction of the superluminal motion and the large-scale jet. Misalignments of several tens of degrees (on the plane of the sky) are observed in other sources^{18,19}, and in 3C273 the large-scale orientation differs by 23° from the core orientation. In this case, the above limit $\theta_j \geq 45^\circ$ may give a fair estimate and we get $L < 3$ kpc (from the maximum width of component 2; see Table 1). The apparent shape of component 2 at different radio frequencies (in our model, $L(\nu) \propto \nu^{-1/2}$) yields $L \approx 1$ kpc. From the latter value we expect that the centroid of component 2 is offset by ~ 0.1 arcs between 1.67 and 15 GHz, consistent with the observed offset 0.1 ± 0.1 arcs (from the contour maps). However, no firm lower limit for L can be derived from geometrical considerations alone. For $\theta_j \approx 45^\circ$, the projected shape and length of the emission region are in excellent agreement with the hydrodynamical models^{8,9}, which predict that the length L should be approximately equal to the hotspot diameter (2.2 kpc; $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is assumed throughout).

The mean free path λ_{ei} limits the maximum number of field reversals in the hotspot region. Thus the observed Faraday depolarization can be used to derive a rather firm upper limit to the density (n_{HS}) of thermal electrons^{20,21}. With an observed depolarization wavelength $\lambda_{1/2} = 0.7 \text{ m}$ (ref. 1) and the mean free path from equation (3) we get:

$$n_{\text{HS}} < 1.5 \times 10^4 \beta_j^{1/2} (B_{\text{nT}} L_{\text{kpc}})^{-1} \text{ m}^{-3} \quad (4)$$

(compare with ref. 21). The upper limit to the jet density n_j (here $n_j = 1/4 n_{\text{HS}}$) leads to further constraints on the magnetic field. From the requirement that the ram pressure of the jet exceed the magnetic field and particle pressure in the hotspot, we get

$$B < [4.2 \times 10^6 B_j^{3/2} L_{\text{kpc}}^{-1} - 1.54 \times 10^4 (1 + \kappa) \beta_j^{-1/3} L_{\text{kpc}}^{-2/3}]^{1/3} \text{ nT} \quad (5)$$

where κ is the ratio of energy stored in protons to that in electrons and equation (2) was used to evaluate the particle pressure term. The kinetic energy flux in the jet should at least balance the synchrotron power emitted. Thus, taking a shock area $A = 4 \text{ kpc}^2$:

$$B < 8.2 \times 10^3 \text{ nT } \epsilon_{\text{syn}} \beta_j^{5/2} (A/4 \text{ kpc}^2) L_{\text{kpc}}^{-1} \quad (6)$$

with the (unknown) synchrotron efficiency $\epsilon_{\text{syn}} = (\text{emitted synchrotron power})/(\text{kinetic energy flux}) < 1$. Equations (5) and (6) restrict the allowed area of the B - β_j plane to $\beta_j > 0.1$ and $30 < B < 100 \text{ nT}$ (for $L < 3 \text{ kpc}$; see Fig. 3).

We may compare our field estimate, equation (2), with the minimum-energy estimate (see ref. 15):

$$B_{\text{min}} = 35 (\kappa + 1)^{2/7} L_{\text{kpc}}^{-2/7} (\nu_b/1 \text{ GHz})^{-1/7} \text{ nT} \quad (7)$$

(Because the spectrum is flat at low frequency the estimate is insensitive to the lower-frequency limit.) The minimum-energy estimate yields hotspot magnetic fields between 24 nT ($\kappa = 0$, $L = 3 \text{ kpc}$) and 120 nT ($\kappa = 100$, $L = 1 \text{ kpc}$), in remarkable coincidence with the result from the break frequency. This supports the assertion that the break is due to the integrated synchrotron emission from a finite region. The estimate in equation (2) is still applicable even if Fermi acceleration is not the relevant mechanism, provided that the general features of the acceleration process are valid (see discussion of assumption (1) above).

On the basis of the (non-relativistic) acceleration model we propose the following parameters for the jet and hotspot 3C273 A:

- Length of hotspot emission region: $L \approx 2 \text{ kpc}$
- Hotspot magnetic field: $B \approx 70 \text{ nT}$
- Jet speed: $u_j \approx 0.3 c$
- Jet density = 1/4 hotspot density: $n_j \approx 60 \text{ protons m}^{-3}$.

The mildly relativistic jet speed would require a high synchrotron efficiency, $\epsilon_{\text{syn}} \approx 25\%$. The Lorentz factors of the electrons near the cutoff and the break are then $\gamma_c = 2.3 \times 10^5$ and $\gamma_b = 700$, respectively. The gyroradius of the highest-energy electrons (10^{-7} pc) is comfortably smaller than their mean free path (equation (3)). The hotspot field is ~ 10 times higher than the minimum-energy field in the jet (8 nT).

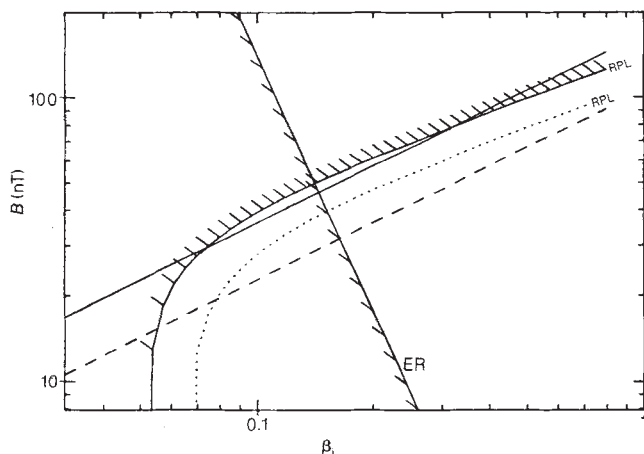


Fig. 3 Magnetic field (B) estimated from the break frequency $\nu_b = 1.5$ GHz for $L = 1.5$ kpc (—) and 3 kpc (---), as a function of the jet speed, $\beta_j = u_j/c$. The parameter plane is restricted by the ram pressure limit, equation (5) ('RPL': —, $L = 1.5$ kpc; ..., $L = 3$ kpc) and the energy requirement, equation (6) ('ER'). Note that the ram pressure limit depends on L , while equation (2) was used to remove the L -dependence from the energy requirement, equation (6). For this figure, the shock area is taken to be 4.0 kpc^2 and $\kappa = 0$.

The most important result of our calculations is that steep-spectrum sources can be naturally explained by Fermi acceleration at a strong, non-relativistic shock. In the case of 3C273, we can account for all of the detailed features of the spectrum. The magnetic field estimated from a break in spectral index $\Delta\alpha = 0.5$ at ~ 1.5 GHz is remarkably similar to that calculated

from the (completely independent) minimum-energy consideration. Our model predicts that the origin of optical synchrotron radiation is confined to a thin (1 pc) layer at the shock discontinuity. An observational test of the model requires accurate maps at two different radio frequencies: the increasing thickness of the emission region with decreasing frequency should result in a significant offset (≥ 0.1 arc s) of the centroid of component 2 and a broadening of its width when observed at, for example, 1.67 GHz and 15 GHz.

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Companion-star beam steering of high-energy particles from Hercules X-1

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A recent model¹ for the 10^{11} – 10^{15} eV γ -ray emission from the 1.24-s X-ray pulsar Hercules X-1 postulates coherent charged particle beams as the photon source, through a cascade process in intervening matter in the binary system HZ Her/Her X-1. This interpretation is complicated by a 1985 observation² of $\sim 10^{12}$ -eV γ -ray pulsations during at least the first hour of the nominal 6-h X-ray eclipse of Her X-1 by HZ Her, which would completely absorb a particle (or photon) beam propagating along or near the line of sight to Her X-1. Here we show that the presence of even a weak dipole magnetic field around the companion star is sufficient to steer such a beam to a suitable target region for producing the observed γ -rays. We demonstrate that models such as that in ref. 1 must include such effects, and we discuss the effects of such beam steering on γ -ray and neutrino observations.

In the past 3 years, six separate detections of distinct 1.24-s pulsations from Her X-1 at energies $\geq 2 \times 10^{11}$ eV have been reported by three groups^{3–5}. The γ -ray emission has appeared as episodes of duration ~ 3 min to ≥ 1 h, with possible correlations to mass transfer between the binary stars, and other unusual X-ray behaviour^{6–8}. The most problematic of these episodes is a 1985 detection reported by the Whipple Observatory group on Mt Hopkins, Arizona, which appears to show strong pulsations occurring during the nominal X-ray eclipse². This ~ 2 h observation spans an eclipse transition at orbital phase 0.932 (eclipse centre = phase 0.0) and continues for ~ 75 min into

eclipse. Pulsed γ -rays appear only after the eclipse ingress, and continue throughout the remainder of the observation ($\sim 20\%$ of the eclipse duration). This and the previous detections therefore suggest an association between intervening matter and the γ -ray emission.

Models for emission of γ -rays of energy $> 10^{11}$ eV from both Her X-1 and X-ray binary Cygnus X-3 have involved the production of γ -rays as secondaries from a particle 'beam dump'⁹. An accretion-fed pulsar electromagnetically accelerates¹⁰ charged particles to produce a coherent beam, which then interacts with the surrounding matter in the accretion disk¹ or even the companion star limb^{11,12}. However, the 10° scattering angle required to explain the γ -rays as cascade secondaries ~ 1 h into eclipse is ~ 3 orders of magnitude greater than the expected mean at ~ 1 TeV.

Thus, both the line-of-sight and limb-scattered trajectories are kinematically very unfavourable. However, with some plausible assumptions concerning the magnetic field of HZ Her, charged particle beams may still account for the reported γ -ray emission. It is unlikely that 1–10-TeV photons can propagate out of the co-rotating region of the pulsar magnetosphere (radius $r \leq 10^8$ cm), because of the effect of pair-production processes, both on the strong magnetic field and the local X-ray photons. A particle beam of > 10 TeV energy would not be greatly attenuated in this manner due to its much higher momentum density; however, the local pulsar field will destroy any phase coherence if the beam must traverse a significant distance within it. For Her X-1, the pulsar magnetopause¹³ is expected to occur at a radius $R_m \approx 5 \times 10^8$ cm, considerably within the pulsar light cylinder at $R = c/\Omega \approx 10 R_m$ (where c is the speed of light and Ω is the pulsar angular frequency), and small compared with the orbital radius of $\sim 4 \times 10^{11}$ cm. Once past this region, the beam may be shielded from the pulsar field, and can thus be steered by the companion-star dipole field.

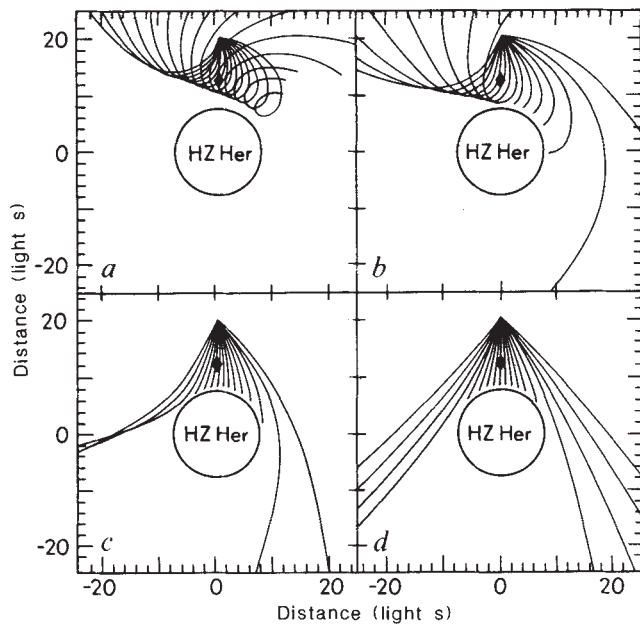


Fig. 1 *a-d*, Proton trajectories in a dipole magnetic field from an idealized binary system patterned after the geometry of HZ Herculis/Hercules X-1. The star's magnetic moment points into the page, and the surface field intensity is taken to be 0.1 G. Proton energies: *a*, 0.5 TeV; *b*, 1.0 TeV; *c*, 5 TeV; *d*, 20 TeV. Her X-1 is at the apex of the trajectories. The inner Lagrange point, $\blacklozenge$, marks the extent of the inner caudal of the Roche lobe of HZ Her (neglected for simplicity here).

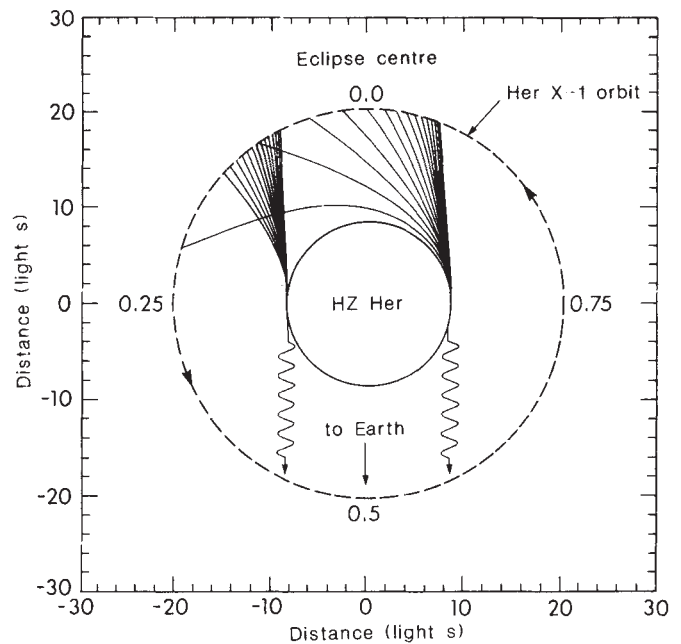


Fig. 2 Proton trajectories from Her X-1 that can produce γ -rays observable at Earth through interaction with the companion star, plotted from their injection point along the orbit of Her X-1 (dashed circle) to the secondary cascade production site at the companion-star limb. The injection energy varies logarithmically for each group of trajectories to the left- and right-hand limb of HZ Her, with a minimum energy of ~ 4 TeV (for the most curved paths) to a maximum shown of ~ 140 TeV (least deflected). This γ -ray emission geometry is chosen to approximately match the parameters required to explain the reported emission of ref. 2.

We model the geometry of an idealized binary system after Her X-1 as viewed from its north pole, with the pulsar and companion star HZ Her orbiting anticlockwise. For simplicity we have taken HZ Her to be spherical, although in fact it is believed to fill its Roche lobe¹⁴. We assume that the interior density profile is described by models for a $\sim 2.2\text{-}M_{\odot}$ star (where $M_{\odot}$ is the mass of the Sun) that has evolved to the base of the giant branch¹⁵. As our line of sight is nearly aligned with the orbital plane of the HZ Her/Her X-1 system¹⁶, the beam must propagate very near to the orbital plane to produce secondaries visible at Earth; thus we assume it lies entirely in the orbital plane.

The instantaneous curvature of the beam is given by the particles' gyroradii in the companion-star dipole field:

$$\Gamma(\rho) = E[ZeB_s(\rho/R_s)^{-3}]^{-1} \quad (1)$$

for a particle energy E and charge Ze , and companion-star surface magnetic field strength B_s and radius R_s . The ρ^{-3} dependence; ρ is the cylindrical polar radius of the particle from the companion star arises from the dipole magnetic field $\mathbf{B}$, with the dipole moment assumed to be antiparallel to the angular momentum of HZ Her. A particle's trajectory may be determined numerically by superposition of a sequence of small arcs with curvature given by equation (1), and radial direction given by the magnetic Lorentz force unit vector

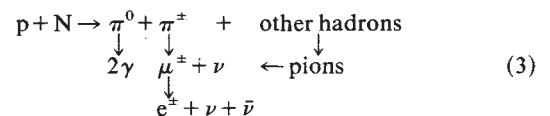
$$\hat{\mathbf{r}} = (\mathbf{v} \times \mathbf{B})[|\mathbf{v} \times \mathbf{B}|]^{-1} \quad (2)$$

for particle velocity $\mathbf{v}$.

Figure 1*a-d* shows the numerically calculated trajectories of protons of various energy as they sweep across the orbital plane with the pulsar rotation. The surface field strength B_s has been taken arbitrarily to be 0.1 G (extrapolated from the ~ 1 -G solar surface field; stellar magnetic fields are undetectable below ~ 100 G). However, these results may be scaled to different field

intensities or particle energies, by keeping the ratio E/B_s constant.

Only particle trajectories that are nearly tangential to the star's surface when they intersect it will encounter the optimal column density for both production¹⁷ and minimal absorption of secondary γ -rays ($\sim 100 \text{ g cm}^{-2}$). This value occurs at depths of $\sim 3\text{--}4 \times 10^4 \text{ km}$ when one integrates along line-of-sight paths through the stellar limb, using an estimated scale height for the atmosphere of HZ Her¹⁶. A typical particle cascade would proceed initially as:



Due to the high secondary particle multiplicity, proton energies > 10 TeV are required to produce ≥ 1 -TeV secondary γ -rays. Fig. 1 shows that, for these field parameters, this is about the minimum primary energy for which pulsations are preserved (with time dispersion < 1.24 s) in the binary system.

Figure 2 shows a sequence of trajectories of particles of varying energy which intersect the limb at the required angle. The orbital phase of their emission is indicated by the intersection of the trajectory with the orbit of Her X-1 (centred around HZ Her, rather than the barycentre, for simplicity). The model predicts γ -ray emission around eclipse ingress and egress, with notable asymmetry in the duration around eclipse centre. The field and the strict geometrical requirements at the beam dump act together as a magnetic time-of-flight spectrometer, with lower energies appearing progressively later. For a power-law primary emission spectrum $N(>E) \propto E^{-\gamma}$, we find $\gamma = 1$ is required for extended emission (> 30 min) after eclipse ingress.

The presence of a multi-TeV particle beam also implies copious neutrino production; however, we do not expect the time-

averaged ≥ 1 TeV neutrino flux to be significantly different from that calculated by others who have not included the effects of a companion-star magnetic field^{18–20}. With reasonable assumptions, the neutrino-to- γ -ray flux ratio could be $\sim 10^2$ – 10^3 , which implies a flux of $\geq 10^{-8}$ cm⁻² s⁻¹ above ~ 1 TeV for neutrinos (using the γ -ray flux of ref. 2). As in models which ignore magnetic steering, the emission will be restricted to orbital phases very near the X-ray eclipse. We summarize observable signatures of magnetic steering in neutrinos and γ -rays as follows:

(1) The low-energy cutoff of charged primaries due to field scattering will result in a similar cutoff in secondary neutrinos and γ -rays. The present lack of detections of Her X-1 in sub-GeV γ -rays supports this claim. The proposed DUMAND (deep underwater muon and neutrino detector) array could confirm this effect in neutrino observations.

(2) The amplitude of the 1.24-s pulsar modulation in the secondary neutrino emission is very sensitive to the primary proton spectrum, because an excess number of low-rigidity primaries with their much greater variance in path length to the companion star will destroy the phase coherence preserved by the high-rigidity particles.

(3) The duration of emission for both neutrinos and γ -rays will be distinctly asymmetric around the eclipse centre, because the Lorentz force does not change sign across the eclipse. The orientation of the companion-star magnetic dipole moment can be determined from this asymmetry, and observations of a change in the energy spectrum with time may be used to analyse the momentum of the primary pulsar beam.

If this model has any validity, then improved TeV γ -ray observations and future neutrino observations, such as with DUMAND, may provide crucial detail on the magnetic fields of binary systems such as HZ Her/Her X-1. Ground-based high-energy observatories could thus generate much otherwise inaccessible information for their relatively modest cost of construction.

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Transitions of viscous fingering patterns in nematic liquid crystals

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Interfacial patterns in a Hele-Shaw cell¹ with no anisotropy are disordered², and in the limit of vanishing surface tension the viscous fingers develop into a fractal structure^{3,4} as a consequence of a cascade of tip splittings. By engraving a grid into the plates of the cell, Ben-Jacob *et al.*⁵ demonstrated the role of a model anisotropy in hydrodynamic systems. Here we introduce an experimental system which uses a nematic liquid crystal as the viscous fluid, so that there is anisotropy in the medium itself. We find that the effective anisotropy⁶ may be tuned by varying the pressure with which the low-viscosity liquid (air, in our case) enters the cell. As a result we obtain re-entrant morphological transitions between random patterns (tip splitting) and quasi-regular patterns qualitatively resembling dendritic growth (stable tips).

A radial Hele-Shaw cell^{1,2} consists of two transparent parallel plates separated by a viscous liquid. Air is injected into the liquid through a hole in one of the plates. Because of the instability of the interface, a complex pattern consisting of finger-like structures develops in the cell. In order to study viscous fingering in nematic liquid crystals, we constructed a radial Hele-Shaw cell from a standard liquid-crystal sandwich structure. The two glass plates measured 70×70 mm; one of them had a 1 mm hole in the centre, with a glass tube connected to it. The sample thickness was 40 μ m. The substance used in the experiment was a eutectic mixture of azoxy compounds labelled with Nematic Phase 7A Licrystal (BDH), which is a standard representative of nematics. The mixture had a wide

nematic temperature range (−10 to +80 °C). Its mean viscosity at room temperature was ~ 30 cP. (The assumption of a newtonian fluid provides a good approximation to the hydrodynamic behaviour of nematic liquid crystals⁷.) In order to achieve higher-contrast patterns, the nematic mixture was coloured by adding 4 wt % of a blue dye (D16, BDH). The pressure was varied by changing the amount of air injected per unit time. We carried out the experiments both with and without initial homogeneous alignment of the liquid crystal by coating the inner surface of the plates with a SiO layer. No effect of the alignment was found.

For low injection rates the interfacial pattern is similar to that obtained using isotropic liquids. Because of the instability of the interface, fingers grow out of the central region. Whenever the radius of the tip of a finger becomes larger than a value characteristic for a given surface tension and applied pressure, the tip splits (Fig. 1a). In the limit of large bubble sizes the cascade of tip splitting leads to a disordered, possibly fractal structure^{3,4}.

On increasing the injection rate, an entirely different pattern is obtained (Fig. 1b, c). The tips are stable and straight main stems with side branches grow out from the centre. The observed pattern is analogous to that of the snowflake-like dendritic crystals produced during non-equilibrium solidification⁸.

When the injection rate is increased still further, the tip splitting mechanism is restored, resulting in the appearance of an irregular interface (Fig. 1d). In this case the patterns obtained are reminiscent of the homogeneous phase recently observed in experiments on diffusion-limited growth^{9–11}. Figure 1 demonstrates that viscous fingering in nematic liquid crystals is qualitatively different from the interfacial patterns in isotropic liquids. If the sample is heated above the nematic-isotropic transition temperature, no morphological phase with stable tips develops, and the interface behaves just as in normal isotropic liquids.

Recent studies of diffusion-limited growth^{8,12,13} indicate that there are two classes of interfacial patterns in such processes: if the anisotropy is weak the tip splitting leads to random structures, whereas for sufficiently large anisotropy regular pat-

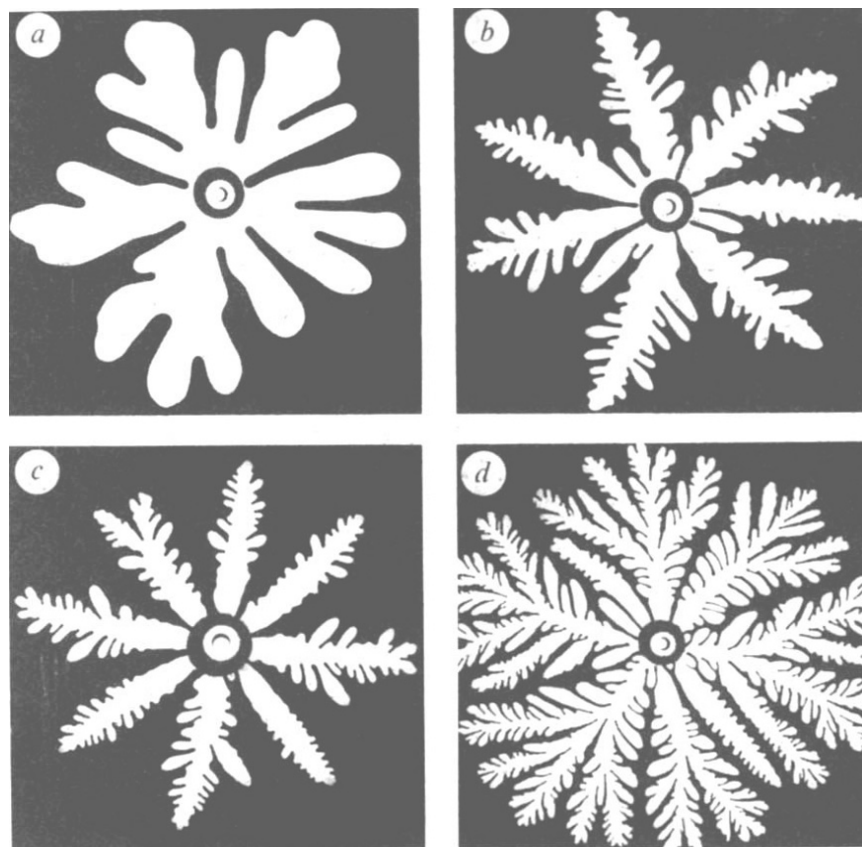


Fig. 1 Interfacial patterns formed in a radial Hele-Shaw cell containing a nematic liquid crystal. As a function of the amount of air injected into the liquid per unit time, I , a sequence of shape transitions can be observed. *a*, For low injection rate ($I = 0.002 \text{ cm}^3 \text{ s}^{-1}$) the structure of the interface is very similar to the irregular patterns obtained in analogous experiments with isotropic liquids. *b*, *c*, On increasing the injection rate the tips become stable, resulting in a dendritic, snowflake-like pattern (*b*, $I = 0.015$; *c*, $I = 0.025 \text{ cm}^3 \text{ s}^{-1}$). *d*, Typical pattern obtained by increasing the injection rate still further ($I = 0.35 \text{ cm}^3 \text{ s}^{-1}$). The tips are again unstable and a disordered structure is produced, which seems to be homogeneous on the length scale of the sample.

terns are formed, because of the stable tips^{6,14-17}. To show that the behaviour we observed is not inconsistent with the present understanding of diffusion-limited pattern formation, we use the concept of effective anisotropy⁶, A_{eff} , defined here for a tip with radius ρ as

$$A_{\text{eff}} = 1 - v/v' = (\rho/v)\partial v/\partial s \quad (1)$$

where v and v' denote the absolute value of the local interfacial velocity at the tip and at a distance ρ from it; s parameterizes the arclength of the interface. Equation (1) expresses the plausible assumption that the inherent anisotropy at the tips manifests itself in the form of a direction-dependent velocity of the interface.

The orientation of the molecules gives rise to the local anisotropy of the surface tension and the shear viscosity, and thus influences $\partial v/\partial s$. On the other hand, ρ and v are mainly determined by the characteristic parameters of the problem, such as average viscosity, surface tension and applied pressure.

The fact that the fingers have stable tips for intermediate injection rates indicates a maximum in the effective anisotropy as a function of injection rate. In what follows we suggest a possible mechanism for the appearance of this maximum.

In liquid crystals, the hydrodynamic flow can orient the director^{7,18}. At low velocities the liquid crystal behaves like an isotropic liquid, because the slow flow does not rearrange the molecules; thus, $A_{\text{eff}} \approx 0$. At higher velocities the mainly radial motion of the interface is fast enough to orientate the director⁹; therefore, $\partial v/\partial s$ becomes relatively large and the anisotropy exceeds the critical value necessary for stabilizing the tips. In our sample we could detect the effect of flow on the director in a region close to the interface by inserting the cell between two orthogonally oriented polarizers. The local anisotropy in the surface tension and viscosity saturates when the molecules are aligned. If the driving force is increased still further, it leads to

a decrease in A_{eff} because, while the velocity in equation (1) continues to increase with the injection rate, there is now no significant change in $\partial v/\partial s$. Thus, at intermediate velocities the effective anisotropy has a maximum, resulting in the re-entrant transition in the pattern structure.

The above model needs further experimental and theoretical verification; our main aim here has been to report the experimental discovery of the morphological transitions shown in Fig. 1*a-d*. We plan to carry out a detailed quantitative investigation of the described phenomenon, including the study of the dependence of patterns on sample thickness, temperature and behaviour close to the nematic-isotropic transition point. Finally we mention that other anisotropic liquids, such as suspensions of rods and platelets, are also expected to exhibit the morphological transitions seen here.

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Radio acoustic measurement of temperature profile in the troposphere and stratosphere

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The radio acoustic sounding system (RASS) uses radar to measure the temperature profile in the atmosphere. In the standard technique of atmospheric radar, the radar backscatter results from electrical permittivity variations due to natural phenomena such as turbulence and precipitation. In the RASS technique, the radar backscatter results from periodical permittivity variations due to density/temperature variations imposed on the atmosphere by an acoustic wave artificially generated in such a way that the acoustic wavelength is half the radar (electromagnetic) wavelength. This 'Bragg condition' is necessary for efficient backscattering. The backscatter echo of the RASS is affected by the Doppler frequency shift arising both from the speed at which the longitudinal acoustic perturbations propagate (the sound speed), and from the radial bulk velocity in the common volume of the atmosphere—the latter can be measured by the standard technique of turbulence scatter. The observed sound speed is reduced to give the local atmospheric temperature. Here we report an experiment using the RASS, carried out on 1–3 August 1985, which consisted of a high-power, very-high-frequency (VHF) Doppler radar at Shigaraki, Shiga, Japan and a movable high-power acoustic transmitter, and which gave the first experimental proof of the possibility of temperature profiling in the troposphere and stratosphere up to an altitude of ~20 km.

Researches on RASS, initiated by Marshall *et al.*¹, have been advanced during the past decade by several groups^{2–4} in various countries, who have concentrated on atmospheric temperature measurements in the planetary boundary layer up to ~2 km above the ground. Most of these experiments used a RASS consisting of an ultra-high-frequency (UHF) Doppler radar and an acoustic transmitter at frequencies ≥ 1 kHz. The altitude ranges for which the temperature measurements in these experiments were successful lie mostly below ~1 km, with an upper limit at 3 km. The present experiment was designed to measure the higher atmosphere, up to the level of the stratosphere. To achieve this, our RASS comprises a monostatic pulsed Doppler radar in the VHF range (the MU radar^{5,6}), completed in November 1984 at Shigaraki by the Radio Atmospheric Science Center of Kyoto University, and a pneumatic acoustic transmitter at frequencies of ~100 Hz provided by the Radio Research Laboratory. Atmospheric absorption of such a low-frequency acoustic wave is less effective, and the acoustic wave may propagate to an altitude of ~30 km without any appreciable attenuation caused by atmospheric absorption. The operational parameters for the MU radar and the acoustic transmitter used in the present experiment are given in Table 1.

For the RASS to attain an efficient radar backscatter from the periodical permittivity variations imposed by an acoustic wave requires that, for monostatic radars, the acoustic wavenumber vector $\mathbf{K}_a$ ($\equiv 2\pi\mathbf{k}_a/\lambda_a$) has to be equal to twice the wavenumber vector $\mathbf{K}_r$ ($\equiv 2\pi\mathbf{k}_r/\lambda_r$) for the primary radar wave; that is, $\mathbf{K}_a \approx 2\mathbf{K}_r$ at the scattering point, where $\mathbf{k}$ is the unit vector of the wave normal and λ is the wavelength, with suffixes *a* and *r* denoting the acoustic and radar waves, respectively. This Bragg condition is equivalent to two conditions: the 'wavelength condition' that $2\lambda_a \approx \lambda_r$, and the 'wave-vector condition' that $\mathbf{k}_a \approx \mathbf{k}_r$, or the angle between the two vectors $\cos^{-1}(\mathbf{k}_a \cdot \mathbf{k}_r) \approx 0$. The power

Table 1 Major operational parameters of the RASS

MU radar (monostatic pulsed Doppler radar)	
Frequency (MHz)	46.5
Antenna aperture (m ²)	8,330
Antenna gain (dB)	33
Beam-width (deg.)	3.6
Peak power (MW)	1
Sub-pulse-width (μ s)	2
Pulse compression (bits bi-phase coding)	8
Pulse repetition (pulses per second)	2,400
Acoustic transmitter (pneumatic acoustic generator)	
Frequency (Hz)	88–102
Acoustic power (W)	100
Antenna	Exponential horn
Antenna gain (dB)	10
Beam-width (deg.)	45
Pulse-width (s)	1
Pulse repetition (pulses per hour)	16

of the backscattered echo is fairly sensitive to both conditions, so that, for the present experiment, the echo power may be reduced to about one tenth of that expected in the ideal case by a deviation in the 'wavelength condition' such that $2\lambda_a/\lambda_r = 1 \pm 0.015$ (where the number of wavelengths in the acoustic pulse $n_a = 100$) or by a deviation in the 'wave-vector condition' such that $\cos^{-1}(\mathbf{k}_a \cdot \mathbf{k}_r) = 0.3^\circ$ (where the distance to the scattering point from the radar site is assumed to be 10 km). It is unlikely that the RASS satisfies both conditions ideally, but it is reasonable to expect that the two conditions can be approximately satisfied.

In the present experiment, the radar wavelength λ_r is constant (at 6.45 m) and the radar wave-vector $\mathbf{k}_r$ is always in the radial direction along the radar beam (beam-width 3.6°), which is steerable in the zenith-angle range between 0° and 30° for the whole azimuth. On the other hand, the acoustic wavelength λ_a and wave-vector $\mathbf{k}_a$ vary along the propagational path under the influence of various atmospheric conditions. The acoustic wavelength at a frequency f_a varies with altitude due to the variation in atmospheric temperature and hence in the local sound speed, $c_s (= \lambda_a f_a)$. Therefore, it is necessary to transmit the acoustic pulses at different frequencies in order to meet the 'wavelength condition' at a wide range of altitudes. In the present experiment, the frequency of the acoustic wave was changed from pulse to pulse in the range between 88 and 102 Hz, with a frequency step of 1 or 2 Hz. The propagational path of an acoustic wave is affected by refraction due to the existence of a vertical gradient in atmospheric temperature, and also by atmospheric bulk motion due to winds and turbulence. A transmitted acoustic wave consists of various rays whose angles of incidence are distributed over a comparatively wide range of directions (the acoustic beam-width for the present RASS is $\sim 45^\circ$). In the simplest case, when there is a vertical gradient in the atmospheric temperature but no atmospheric bulk motion, the acoustic ray of vertical incidence may satisfy the 'wave-vector condition' in association with the radar beam of vertical incidence, provided that both waves are transmitted from the same place. The acoustic rays of oblique incidence refract upwards, and their paths are no longer in the radial direction from the transmitter. When there are horizontal winds as well as a vertical gradient in temperature, it is necessary to choose a place for the acoustic transmitter on the windward side of the radar antenna and to vary the direction of the radar beam until it satisfies the 'wave-vector condition' at the altitude of interest. In the present experiment, the acoustic transmitter was moved to three appropriate positions within a few hundred metres on the windward side of the radar antenna, and the radar beam direction was swept in the zenith-angle range between 0° and 10° on both the windward and leeward sides, so that RASS

Fig. 1 Contours of RASS echo power spectra in the coordinates of height (resolution 300 m) versus Doppler velocity V_d (m s^{-1}) (resolution 0.6 m s^{-1}) and reduced temperature T ($^{\circ}\text{C}$) (resolution $\approx 0.9^{\circ}$). A train of blobs in the spectral contours reproduced by a superposition of the data from individual soundings indicates the RASS echoes which are caused by acoustic pulses at discrete frequencies of 101, 99, 97, 95, 94, 92, 90, 89 and 88 Hz.

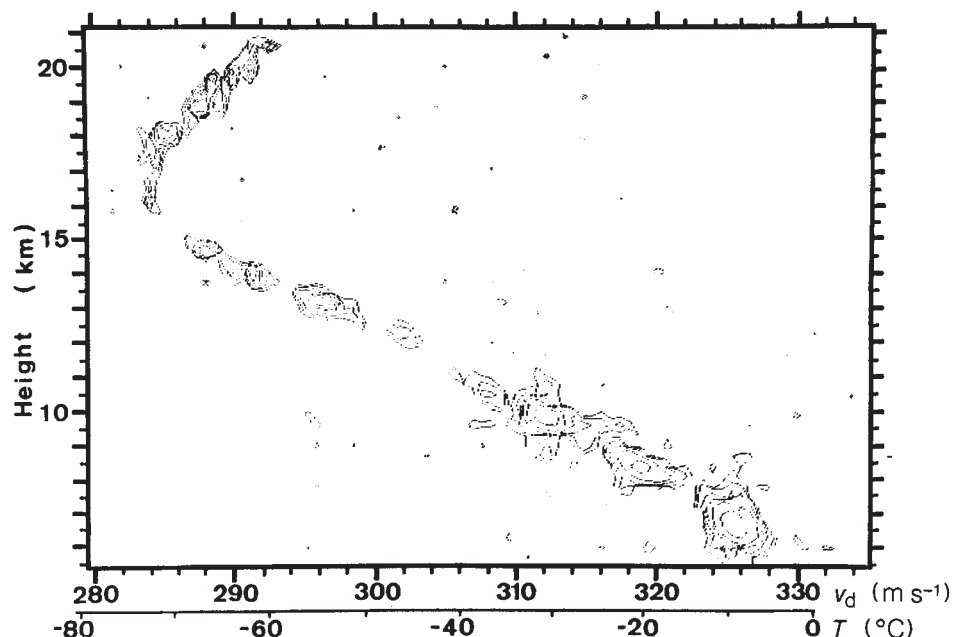
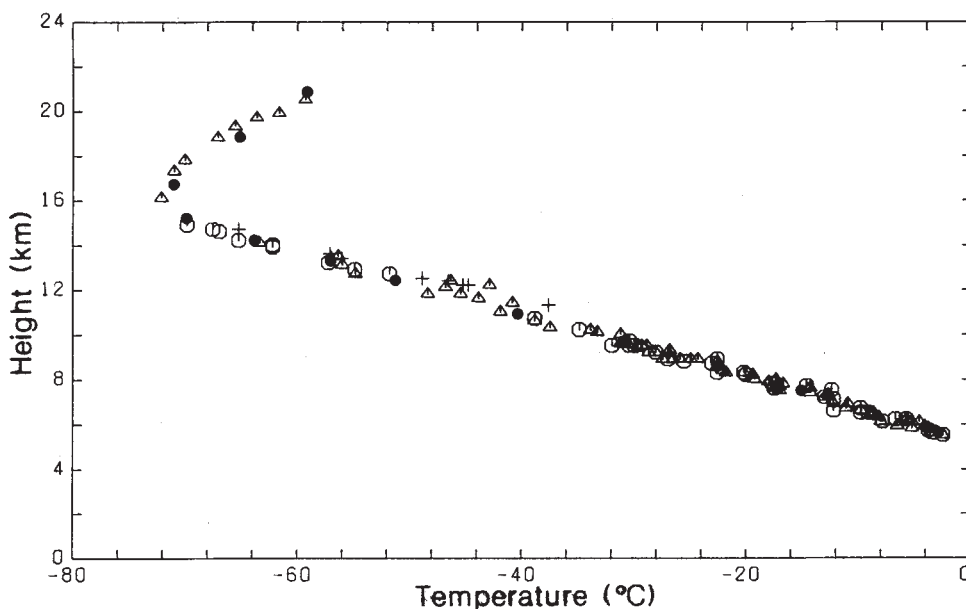


Fig. 2 Temperature profile reduced from all of the RASS echoes observed on 1–3 August 1985 at three different radar-beam zenith angles ($\chi=0^{\circ}$ ($\circ$), 5° (Δ) and 10° ($+$)), where the height is corrected to the value above sea level and the temperature is reduced from the Doppler velocity excluding the wind component. $\bullet$, Temperature profile from the radiosonde measurements at Shionomisaki.



echoes could be obtained from the troposphere and stratosphere.

Examples of the Doppler power spectra acquired from the RASS experiment on 1–3 August 1985 are shown in Fig. 1, where a train of blobs in the spectral contours is reproduced by a superposition of the data from individual soundings. Each blob from right to left indicates the RASS echo caused by passage of the acoustic pulse at each of the nine discrete frequencies, 101, 99, 97, 95, 94, 92, 90, 89 and 88 Hz. The power spectra were obtained by means of fast Fourier transform analyses with an integration time of 3 s, Doppler frequency resolution of 0.2 Hz or Doppler velocity resolution of 0.6 m s^{-1} , and height resolution of 300 m. In Fig. 1, the temperature is reduced from the Doppler velocity V_d (m s^{-1}) by the relation $T(^{\circ}\text{C}) = V_d^2/(\gamma R/M) - 273.2$ (assuming a dry atmosphere without wind), where γ is the ratio of the specific heat at constant pressure to the specific heat at constant volume, R is the universal gas constant ($8.314 \times 10^3 \text{ J mol}^{-1} \text{ K}^{-1}$) and M is the mean molecular weight of the atmospheric gas. The temperature error arising from the pres-

ence of water vapour in the atmosphere is $\leq 1^{\circ}\text{C}$. The ratio of the peak echo level at the centre of each blob in Fig. 1 to the background noise level is estimated to lie in the ranges 27–20 dB, 18–10 dB and 14–11 dB, for the groups of the blobs belonging to the altitude ranges $<10 \text{ km}$, 10–15 km and 15–20 km, respectively. The peak level as well as the spectral pattern of the RASS echo blob varies from blob to blob. The Doppler frequency arising from the speed of sound, $f_d (= 2\lambda_a f_a/\lambda_r)$, measured at the centre of each blob is not always equal to the frequency f_a of the acoustic wave causing each of the RASS echoes. These facts suggest that the power spectra of the RASS echo is under the influence of a delicate balance between the effects of the 'wavelength condition' and the 'wave-vector condition', and that the perfect 'wavelength condition', $2\lambda_a = \lambda_r$, is not necessarily satisfied at the centre of the RASS echo blob. Therefore, the Doppler technique is effective for an accurate measurement of the sound speed. In addition, the fine frequency resolution by the Doppler technique results in an efficient detection of the RASS echo, which has a higher signal-to-noise ratio.

In Fig. 2, the central heights of all the blobs in the power spectra of the RASS echoes obtained on 1–3 August 1985 are plotted against temperature, where the height is corrected to that above sea level and the temperature is reduced from the measured Doppler velocity V_d by the relation, $T(^{\circ}\text{C}) = (V_d - W \sin \chi)^2 / (\gamma R / M) - 273.2$, where the effect of horizontal wind in the direction of the radar beam azimuth, W (m s^{-1}), measured from the standard technique of turbulence scatter, are compensated and χ is the zenith angle of the radar beam. The temperature profile from the present RASS experiment agrees reasonably well with the average temperature profile from the radiosonde measurements by the Japan Meteorological Agency at Shionomisaki, ~ 157 km south of the Shigaraki radar site (see Fig. 2).

Comparison between the vertical thermal profiles measured by the present RASS and by the radiosonde system demonstrates that the RASS can measure thermal profiles up to an altitude of at least ~ 20 km with an accuracy and vertical resolution comparable with the radiosonde technique, with the additional advantages of a possible rapid succession of measurements and a comparatively low cost of operation. The RASS technique effectively complements the capabilities of VHF radars, such as the measurement of winds, waves and turbulence, and other attempts to retrieve temperature profiles from such radar measurements by using the measured Brunt–Väisälä frequency.

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Atomic structure of ultrafine catalyst particles resolved with a 200-keV transmission electron microscope

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Small atomic assemblies (diameter 10–100 Å) possess several unusual physical and catalytic properties^{1–9}. Speculations regarding the crystalline nature of individual particles of supported metals dispersed to this degree, as well as colloidal metallic sols, date back to Faraday's researches on divided metals. Interest has been renewed following the demonstration^{10,11} that metals could readily be prepared in colloidal form. Similarly, binary semiconductors such as CdS exhibit novel properties which are of potential value in photoelectrochemistry. Thus, the electronic band-gap of bulk CdS is 2.4 eV, whereas a CdS cluster of $\sim 18^{\circ}$ diameter—corresponding to one of the 'magic agglomeration numbers'—has a band-gap closer to 3.4 eV. Moreover, the conduction-band edges of such ultrafine semiconductors functioning as microelectrodes are sufficiently shifted to favour the photoreduction of H^+ (as

gaseous H_2) from water⁴. Reliable methods are needed to probe the ultrastructure of very fine particles, especially as such methods can elucidate the nature of many other ultrafine materials. Examples of such materials are: (1) bimetallic systems⁸, such as Pt/Ir on oxide supports for the catalytic re-forming of hydrocarbons to improve octane ratings; (2) semiconductors such as TiO_2 overlaid with comminuted Pt for the photocleavage of water^{12,14}; (3) dispersed Rh or Pt on oxide supports for controlling automobile exhausts¹⁵; and (4) large clusters of transition metal and noble metals of the type $\text{Au}_{55}[\text{P}(\text{C}_6\text{H}_5)_3]_{12}\text{Cl}_6$, which bridge the gap between molecular and extended metallic structures⁶. High-resolution electron microscopy is a possibility as it probes structures directly in real-space^{16–18}. Very-high-voltage transmission electron microscopes (TEM), operating between 400 and 1,500 keV, would provide the best interpretable resolution; however, they cannot usually be fitted with *in situ* micro-analytical facilities based on X-ray emission—essential for sample characterization of mixed phases—and voltage stability is harder to achieve as accelerating voltage increases. Here we report results obtained with a modified commercial 200-keV TEM. A new type of side-entry specimen stage permits the investigation of atomic arrays in minute assemblies of Au and in colloidal catalysts of Pt, such as those used in photoredox processes^{4,19,20}. These are shown to be crystalline but relatively rich in disordered regions, both within their bulk and on their surface.

Resolution of atomic structure in the electron microscope is a complex problem, and to compare lens characteristics it is necessary to consider the so-called phase contrast transfer function²¹. This is a measure of the relative phase shift of the electron beam passing through the objective lens as a function of deviation from the optic axis. This function is conventionally expressed as $-\sin \chi$, where χ is given by:

$$\chi = \frac{2\pi}{\lambda} \left\{ \frac{1}{2} \Delta F \alpha^2 - \frac{1}{4} C_s \alpha^4 \right\}$$

where λ is the electron wavelength, C_s the coefficient of spherical aberration, ΔF the deviation in focus from the Gaussian image

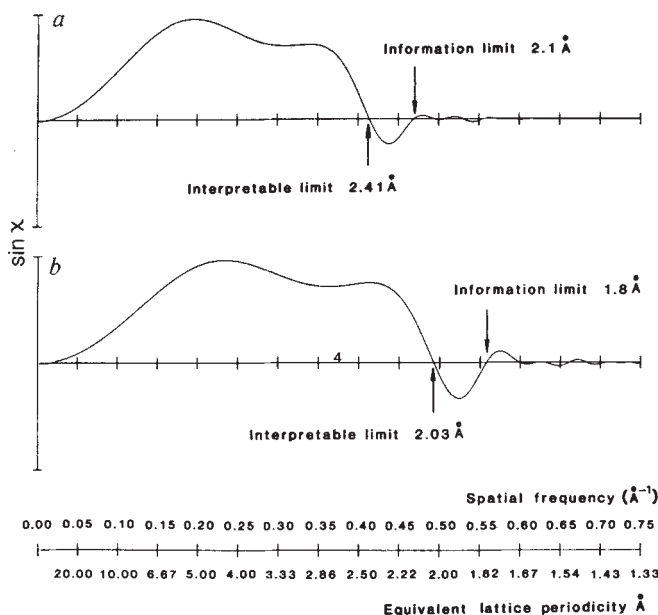


Fig. 1 *a*, Phase contrast transfer function ($-\sin \chi$) for the standard version of the JEOL-200CX microscope. The lens parameters are: spherical coefficient (C_s), 1.2 mm; focal length (f), 2.0 mm; beam divergence, 1 mrad; defocus (ΔF), 650 Å. *b*, The equivalent values for the modified instrument are: C_s , 0.6 mm; C_c , 1.05 mm; f , 1.6 mm; ΔF , 460 Å; beam divergence, 1 mrad. The interpretable resolutions and information limits are shown.

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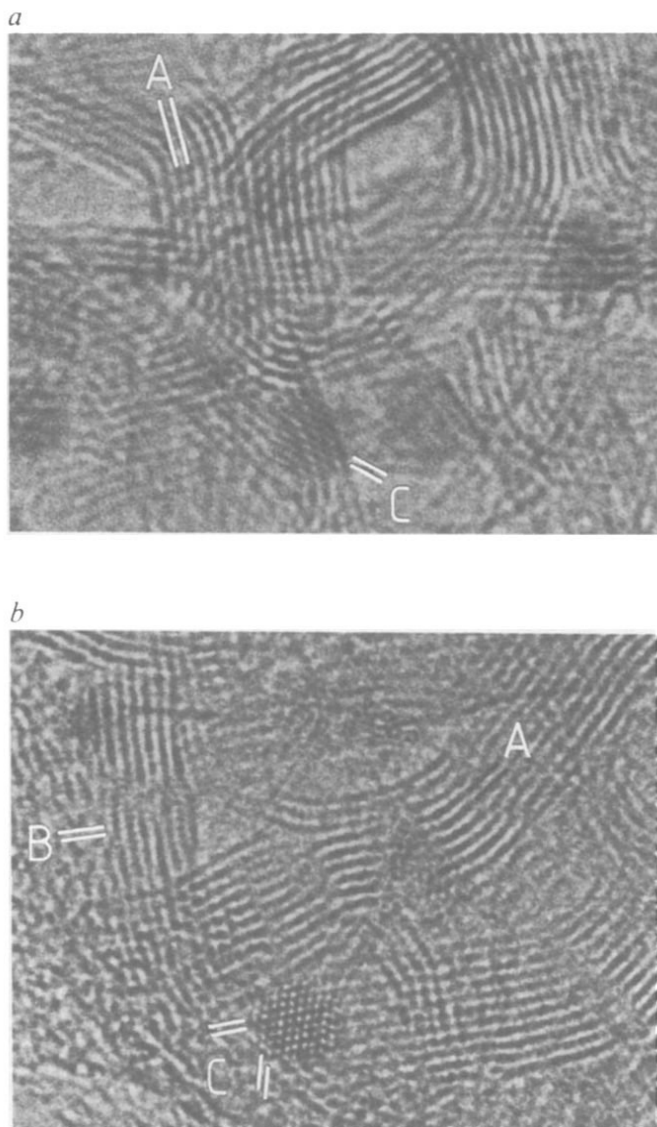


Fig. 2 *a*, High-resolution electron micrograph of Pt particles on a graphitic carbon support, taken with the standard instrument. The 3.4-Å spacings of the support are shown at A, with the 2.26-Å {111} spacings of Pt in the lower particle at C. *b*, Different region of the same specimen, imaged with the modified instrument. In addition to the fringes at A and C, the 2.1-Å {100} spacing of graphite is also resolved at B. Note that in the Pt particle at C, two sets of {111} spacings are visible, identifying the orientation as {110}. (In both cases, the imaging conditions are not necessarily at the optimum ΔF values given in Fig. 1.)

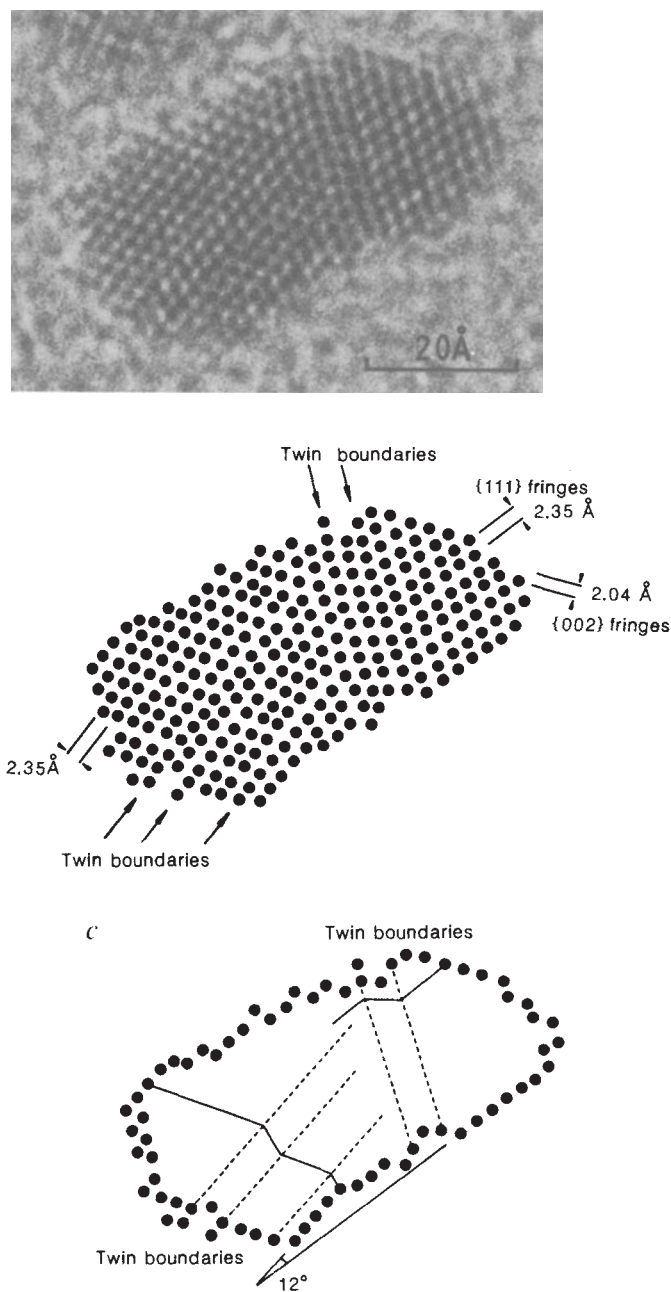


Fig. 3 *a*, High-resolution electron micrograph of an Au particle (prepared by evaporation onto amorphous Si) taken with the modified instrument, along {110}. The ΔF value is estimated to be close to the optimum value. *b*, Schematic representation of *a*, showing the various interplanar spacings and the position of micro-twin boundaries. *c*, More general outline of the micro-twin regions, showing the 12° misalignment between two parts of the crystal.

plane and the scattering angle α ($=1/d_{(hkl)}$) is a measure of the deviation from the optic axis of the objective lens. This function is shown in Fig. 1*a* for the JEOL-200CX TEM, where $C_s = 1.2$ mm. $\sin \chi$ varies with ΔF , and is here shown at the optimum ΔF value, where the effects of ΔF and C_s oppose one another over the largest possible range of values of α . The relative phase shift is approximately the same for all image periodicities from 20 Å down to 2.41 Å. Accordingly, this latter value is defined as the 'interpretable point resolution' of the instrument.

Periodicities smaller than 2.41 Å can still be resolved, and the {111} and {200} spacings of metallic Au (2.35 and 2.02 Å respectively) have been observed in the JEOL-200CX and other instruments operating at lower voltages¹⁶, but their relative phase shifts are in a different sense from those above 2.41 Å. In a bulk sample of perfectly crystalline metal, this presents no problem, as all the image detail is contained in the {111} and {200}

periodicities, but in a small metallic aggregate, which is essentially an aperiodic object, it is important that all periodicities down to that of {200} have the same relative phase shift, otherwise the true atomic structure will not be correctly imaged. The ultimate limit of resolution of an electron microscope, the so-called 'information limit', is set by the combined effect of electron beam, high-voltage and lens current instabilities, coupled with the finite divergence of the beam itself, and is usually indicated by a damping 'envelope' applied to the phase contrast transfer function. In Fig. 1*a* this information limit occurs at a value of α corresponding to 2.1 Å, so that no structural detail of spacings smaller than 2.1 Å can be imaged at any value of

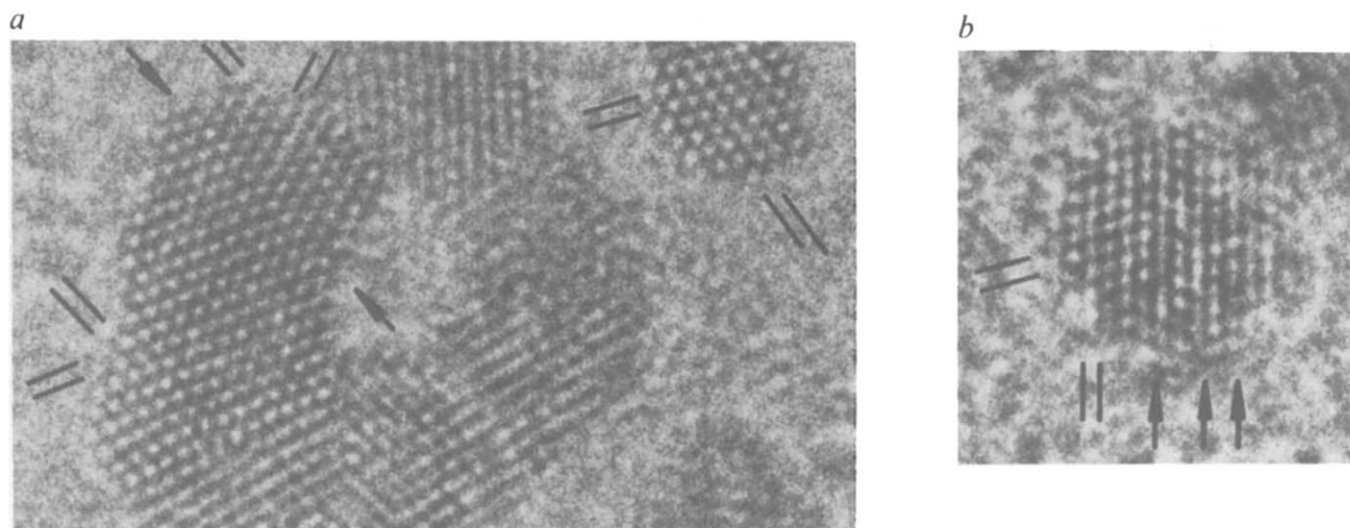


Fig. 4 Catalytically active colloidal Pt particles imaged with the modified instrument. *a*, An agglomeration of several particles, with a micro-twin boundary (arrowed) in the largest aggregate; *b*, a single particle with three twin boundaries. In both cases, the 2.26-Å spacings are indicated by pairs of lines. (The ΔF value is somewhat greater than the optimum.)

ΔF . (Note that in a real object, where dynamical effects obtrude, the phase contrast transfer function is not the only factor affecting resolution. These effects are, however, specimen-dependent; thus, when lens characteristics are compared, the transfer function is the only valid criterion.)

As there was, until now, no way of extending the performance envelope of TEMs operating at 200 keV, the satisfactory imaging of metallic particles required the use of instruments operating at 400 keV and beyond, where the smaller electron wavelengths permitted the use of smaller deviations of diffracted beams from the optic axis^{22,23}. However, by employing a new type of objective lens pole-piece with greatly reduced dimensions, coupled with a new type of specimen stage, we have achieved a significant improvement in the performance envelope of the JEOL-200CX TEM using the available lens power supplies. The phase contrast transfer function for this new lens (Fig. 1*b*) shows that the interpretable point resolution now extends to 2.03 Å, with an information limit of ~ 1.8 Å. The parameters for this new lens calculated using the finite-element method²⁴ are $C_s = 0.6$ mm; C_c (chromatic aberration coefficient) = 1.05 mm and focal length = 1.6 mm. These values have been verified experimentally using standard procedures²⁵. The reduction in pole-piece dimensions necessitates an extremely thin specimen holder, which can be satisfactorily accommodated in the new specimen stage. Full details will be published elsewhere. In addition to the improvement in resolution, the condenser-objective nature of the new lens results in the ability to generate focused beams of smaller dimensions (~ 400 Å diameter) than in conventional systems.

Finely divided Pt particles on a support of graphitic carbon were prepared by impregnation with K_2PtCl_6 in ethanol, followed by reduction in H_2 at 250 °C, and were imaged in the JEOL-200CX before and after modifying the pole-piece and stage as outlined above. The significant improvement in resolution, and hence in retrievable structural information, both of the catalyst particles and of the supporting graphitic carbon, can be seen by comparing Fig. 2*a* and *b*. In Fig. 2*a*, taken with the standard specimen stage and pole-piece, the 3.44-Å {002} fringes of the graphitic carbon are easily resolved, and Pt particles, if in the correct orientation, show the 2.26-Å {111} fringes. Note, however, that the overall shape of the Pt particles is very imperfectly imaged and the general definition is poor. With the modified arrangement, shown in Fig. 2*b*, the differences are very marked. Not only are the 2.26-Å spacings of the Pt particles

resolved much more clearly, indicating that it is in a {110} orientation with respect to the electron beam, but the 2.1-Å {100} fringes in the graphitic carbon support are also visible. Furthermore, the outline of the Pt particles is better defined and details of their surface structure are just about visible.

The power of the modified TEM is also illustrated in Fig. 3, where a fine particle (cross-sectional area $\sim 6 \times 10^3$ Å²) of Au evaporated onto an amorphous Si support film is imaged down a {110} direction. The atomic detail (Fig. 3*a*) is very clearly resolved, and it is also apparent that the particle contains micro-twin boundaries. Topographical irregularities (on the atomic scale) of the particle surface are also relatively pronounced, there being numerous steps and re-entrant gaps of atomic dimensions. These are shown schematically in Fig. 3*b*, *c*. In general, however, the particle is crystalline, although there are incompletely explained features, such as the deviation of $\sim 12^\circ$ in the orientation of the atomic arrays on one side of a twin boundary (Fig. 3*c*).

The ability of a Pt colloidal sol, prepared by citrate reduction of H_2PtCl_6 and stabilized against flocculation²⁶, to function as a catalyst for the reduction of water to H_2 has recently been described²⁷. (Colloidal Pt sols rival blood catalase in the catalytic decomposition of H_2O_2 , a fact established by Bredig at the turn of the century¹¹.) Colloidal metal catalysts have recently been shown²⁸ to be very active in the rearrangement of hydrocarbons (see also ref. 11). In the sols we have studied, the final concentration of Pt metal was 5×10^{-4} mol dm⁻³.

Our improved microscope again made it possible directly to probe the structure of these Pt sols (mounted on an amorphous carbon film). From typical micrographs such as those shown in Fig. 4*a*, *b*, we observe that the interplanar spacings, atomic environments and degree of translational symmetry are all as expected of crystalline bulk Pt. The sol particles are crystalline, but they show a greater tendency to exhibit micro-twinning than do small particles of Pt prepared by reduction of solvent-deposited platinum chloride. As with sputtered Au, these ultrafine particles also show considerable surface roughness (to an extent reminiscent of the structure postulated by H. S. Taylor²⁹ for active sites in Ni catalysts). In the context of photoelectrochemistry, it is significant that the topography of the outermost layers and the regularity of those immediately below the surface are essentially the same as those of macrocrystalline Pt.

We are confident that the performance of 200-keV TEMs can be improved still further. Values of C_s close to 0.50 mm are

possible, taking the information limit to $\sim 1.6 \text{ \AA}$, well suited for the elucidation of the physics and chemistry of many kinds of ultrafine particles, which have widespread use in fields other than heterogeneous catalysis.

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Energy conversion catalysis using semiconducting transition metal cluster compounds

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Semiconductors containing transition metal clusters within their crystal lattice have recently been proposed as catalysts for multi-electron transfer reactions. This is because of the expected action of the clusters as reservoirs for electronic charge carriers and sites for cooperative electron transfer at a reasonably constant electrochemical potential^{1,2}. Here we report the first test of the capacity of such materials for oxygen reduction in an acid medium, for operation in fuel cells. The compound $\text{Mo}_{4.2}\text{Ru}_{1.8}\text{Se}_8$, which contains octahedra statistically composed of 4.2 molybdenum and 1.8 ruthenium atoms, turned out to have a catalytic behaviour comparable to platinum, which has so far been the best catalyst for fuel cells. The material costs of this new type of catalyst amount to only a few per cent of those of platinum.

Energetically interesting electrochemical or photoelectrochemical reactions (such as the photoelectrolysis of water, oxygen reduction in fuel cells and (photo)reduction of CO_2 or N_2) all involve the transfer of several electrons to intermediates with variable redox potentials and kinetic parameters. For optimum catalytic performance, intermediates should bind to the electrode in such a way as to approach the thermodynamic redox potential of the overall reaction as closely as possible. The problem of solar generation of fuels has been approached by identifying semiconducting materials that provide a high density of transition metal *d*-states bordering the forbidden

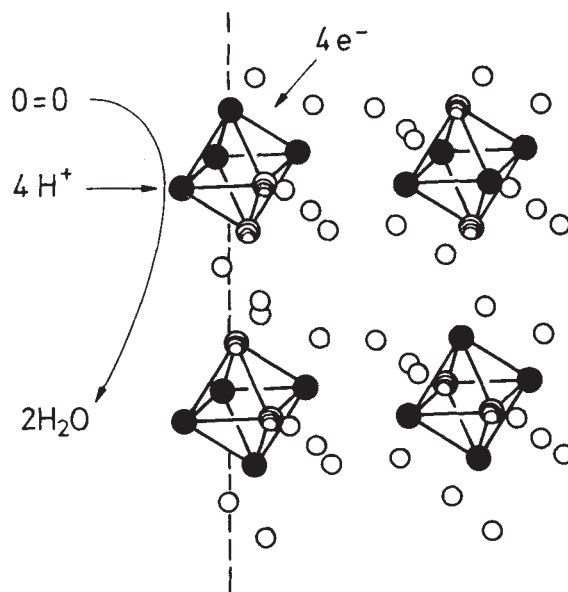


Fig. 1 Schematic interaction of dissolved molecular oxygen in an aqueous electrolyte with the surface of a $\text{Ru}_{1.8}\text{Mo}_{4.2}\text{Se}_8$ semiconducting cluster compound. ●, Mo; ○, Ru; ⊙, Se.

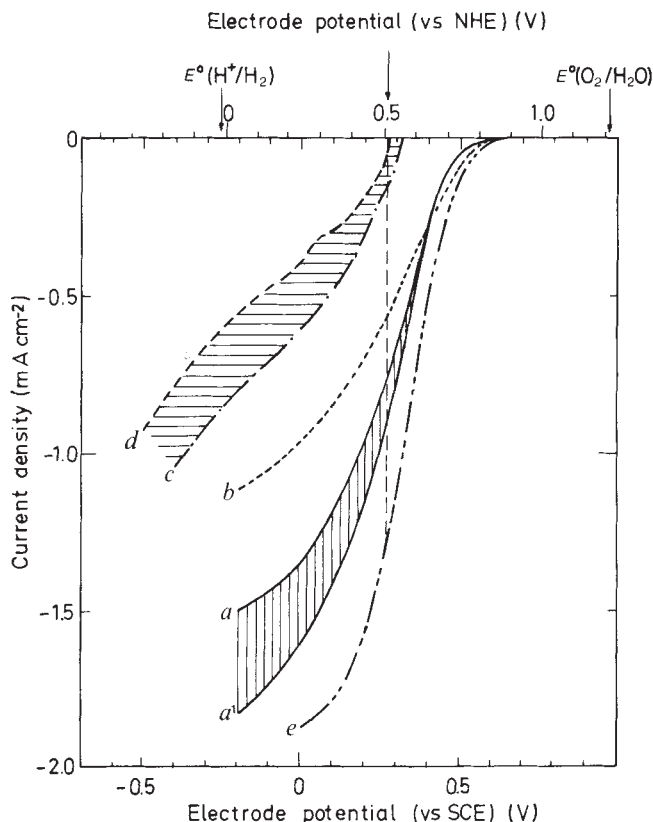


Fig. 2 Catalytic effect of oxygen reduction on several cluster compounds and Pt in 0.5 M H_2SO_4 at $\omega = 400 \text{ r.p.m.}$, $v = 5 \text{ mV s}^{-1}$. a, $\text{Mo}_{4.2}\text{Ru}_{1.8}\text{Se}_8$; b, $\text{Mo}_{3.7}\text{Ru}_{2.3}\text{Se}_8$; c, d, $\text{Ni}_{0.85}\text{Mo}_6\text{Te}_8$; e, Pt. At the top are shown the thermodynamic potentials of H_2 and O_2 for this medium ($\text{pH} = 0.3$). The arrow placed at 0.51 V/NHE (overpotential = 0.7 V) indicates the working conditions in a fuel cell. c, d, Electrodes with less and more Ni at the surface, respectively (as revealed by scanning electron microscopy and X-ray microanalysis). NHE is the normal hydrogen electrode, SCE is the saturated calomel electrode.

Table 1 Kinetic data for O₂ reduction in 0.5 M H₂SO₄

Catalyst	(E/log i)§ (-mV per decade)	$B \times 10^3 / (\text{mA r.p.m.}^{-1/2})^*$ $n = 4e^-$		$k \text{ (cm s}^{-1})^*$ $n = 4e^-$		$I_R / I_D / N$
		Calculated	Experimental	0/V vs SCE	-0.2/V vs SCE	
Mo _{4.2} Ru _{1.8} Se ₈	100	2.68	2.6	0.40	0.58	0.028–0.039
Mo _{3.7} Ru _{2.3} Se ₈	100	2.37	2.0	0.22	0.32	—
Ni _{0.85} Mo ₆ Te ₈ †	208–215	2.67	2.1 ± 0.3	—	0.15	—
Ni _{0.85} Mo ₆ Te ₈ ‡	263–273	2.67	2.6 ± 0.4	—	0.08	—
Pt	125	3.4	4.0	0.54	—	0.04

* The following parameters were used in the calculation: oxygen concentration, $C_{O_2} = 1.1 \times 10^{-6} \text{ mol cm}^{-3}$; diffusion coefficient, $D_{O_2} = 1.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, kinematic viscosity, $\nu = 0.01 \text{ cm}^2 \text{ s}^{-1}$ (ref. 9).

† Less Ni; ‡ more Ni in electrode surface (as revealed by scanning electron microscopy and X-ray microanalysis).

§ E is electrode potential, i is current density.

energy region (d-band semiconductors). During a detailed study of RuS₂, the best material available for photo-induced evolution of oxygen³, it became apparent that ~0.5 eV of energy per transferred electron is lost in the course of trapping positive charge carriers in the interfacial transition metal complex, which is probably a peroxo-type complex of ruthenium with oxygen. We decided that a reasonable way of overcoming this problem of catalysis and energy loss would be to replace individual reactive transition metals in the semiconductor lattice by clusters of transition metals^{1,2}.

The first semiconducting cluster materials available, Mo_{4.2}Ru_{1.8}Se₈, Mo_{3.7}Ru_{2.3}Se₈ (derived from metallic Chevrel phases by substituting Ru for Mo)⁴, turned out to have excess charge carrier concentrations and only small photoeffects ($E_g = 1.3 \text{ eV}$). The catalytic study was therefore not performed on photoevolution of oxygen from water, but on the reverse reaction, the reduction of oxygen in the dark. Figure 1 shows the crystal and supposed interfacial structure of Mo_{4.2}Ru_{1.8}Se₈, the best-performing material, in a statistical sense.

The most attractive medium for oxygen reduction for the purpose of energy conversion in a fuel cell is an acid electrolyte. The most efficient catalyst in such an environment is platinum^{5,6}. We have therefore concentrated on comparing the oxygen reduction performance of the selected transition metal cluster compounds with that of platinum. Rotating disk (RDE) and rotating ring disk (RRDE) electrodes were employed. The comparison shown in Fig. 2 reveals that the catalytic properties of Mo_{4.2}Ru_{1.8}Se₈ for oxygen reduction (the reduction curves are in the dashed area) come close to that of Pt (checked for consistency with literature data). At an overpotential of 0.7 V, where oxygen reduction in fuel cells typically operates, the cluster compound, which has not yet been optimized with respect to material properties, is inferior to Pt by only 30–40%.

A summary of kinetic parameters (discussed in more detail elsewhere, together with surface analytical and material data⁷), is presented in Table 1. From these data, Tafel plots (electrode potential versus log current density, corrected for mass transfer) indicate that a primary electron transfer is rate-determining for the semiconducting clusters of (Mo, Ru)₆Se₈ (the theoretical value is -118 mV per decade at room temperature). The behaviour of the Ni-containing compounds appears to be complicated by complementary reactions (such as adsorption).

The calculated data for Levich slopes, B , agree quite well with those obtained experimentally for a direct four-electron reduction of oxygen to water. To assess this hypothesis for our best catalyst (see Fig. 2) we have undertaken RRDE experiments, using the ring current response to monitor hydrogen peroxide production. The ratio of ring, I_R , to disk, I_D , currents, normalized for the collection efficiency, N , of the ring, therefore defines the relative contributions of the four-electron process (to water) and two-electron process (to H₂O₂). As shown in Table 1, only 3–4% of the transferred charge yields hydrogen peroxide, similar to the case of platinum. The catalytic activity of the investigated Mo_{4.2}Ru_{1.8}Se₈ compound proved to be stable

in laboratory experiments and X-ray photoelectron spectroscopy measurements did not indicate a change of surface composition. The activity was maintained during subsequent repetitive operations.

The catalytic behaviour is strongly dependent on the transition metal stoichiometry within the cluster as seen with the cluster compound Mo_{3.7}Ru_{2.3}Se₈, which is catalytically less active (Fig. 2). The difference can also be seen in the rate values, k , obtained assuming four-electron transfer for the different materials at the same potential (Table 1). When a metal, such as Ni, is inserted between the Mo₆ octahedra as in Ni_xMo₆Te₈ ($x = 0.85$) the catalytic activity is even lower. This demonstrates the active catalytic role of the transition metal cluster (octahedron). The fact that Mo and Ru as well as their oxides, studied separately, are not catalytically useful for oxygen reduction⁸ is further evidence. It must therefore be the cooperative action of the transition metals within the cluster that determines the catalytic activity. Recent studies in our laboratory, in which finely ground particles of the cluster material were embedded into a thin Nafion film covering a p-type semiconductor Gallium phosphide electrode, showed a clear improvement of photo-induced hydrogen evolution. This demonstrates the advantage of using transition metal cluster compounds for the generation of solar fuels.

The estimated cost of the cluster compound (~4% of Pt) was obtained by taking into account the price of the pure elements and the density of the compound, and by considering a production technique in which the elements are simply mixed together at elevated pressure and temperature (for example, $P = 0.7 \text{ GPa}$, $T = 1,470 \text{ K}$)⁴.

We believe that it is no coincidence that transition metal clusters have evolved in biological multi-electron transfer (examples are the co-factor of nitrogenase, four-centre ferredoxin and the manganese centre for photosynthetic oxygen evolution). We expect that successful future synthetic energy conversion materials will show similar transition metal clusters, although in a much simpler inorganic framework structure. Major research efforts on the material and interfacial science of synthetic cluster compounds are necessary to substantiate these expectations.

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Frontal surveys with a towed profiling conductivity/temperature/depth measurement package (SeaSoar)

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In April 1986, RV *Oceanus* participated in the Frontal Air-Sea Interaction Experiment (FASINEX¹) in the region 27–29° N, 68–71° W. The objective was to survey a pronounced oceanic front^{2–4} and its effect on the oceanic and atmospheric boundary layers. Although moorings have yet to be recovered, and much of the data analysis is as yet only preliminary, it is possible to report here the typical density structure of a front as derived from SeaSoar^{5,6} (Fig. 1a) observations, which were analysed within a few hours of collection. Previously unreported detail is revealed, including subducted pycnostads (areas of weak vertical density gradient) with anomalous temperature/salinity signature, splitting of the frontal jet and three-dimensional structure not apparent in the surface temperature field.

Oceanic fronts^{7,8} have cross-frontal scales down to 10 km (less than the Rossby radius of deformation), and develop along-front meanders on scales of 50–100 km which change within a few days⁴. Surveys with conventional lowered conductivity/temperature/depth sensors (CTD) cannot resolve these scales. The SeaSoar, however, resolves the small spatial scales well, with casts every 1–3 km (Fig. 1b). It is harder to resolve both the (100 km)² meander scale and the 3-day timescale, because the 4 m s⁻¹ (8 knot) towing speed allows a track only 1,000 km long to be surveyed in 3 days. Two kinds of survey were carried out in FASINEX. To resolve temporal evolution, a box 30 km by 80 km was surveyed four times in 4 days. To survey a meander, a grid survey with eight north-south tracks 16 km apart was carried out over 3.4 days (Fig. 4); this survey is described here.

Figure 2 is a cross-frontal section from the grid survey, part of Leg 4 in Fig. 4a. Eastward geostrophic velocities relative to the 250 dbar level are contoured in Fig. 3 for Legs 1 and 4. Properties mapped on constant pressure or density surfaces for the whole survey are shown in Fig. 4. The sections shown are typical of about 30 sections made during the four-week cruise. Recurrent features (identified by letters on the figures and in the text) are summarized below.

A 100-m thick mixed layer is broken by the frontal outcrop (A; Figs 2a, b, 4a, at which a density/temperature jump of 0.5 kg m⁻³ and 1.5 °C occurs across 15 km. The isopycnal slope through the mixed layer lies in the range $(2-6) \times 10^{-3}$. Modulations of the mixed-layer depth of up to 50 m are controlled by the front. Beneath the surface outcrop, a pycnostad (B; Figs 2b, 3a) of mixed-layer water from north of the front extends to 140 m depth. The most steeply sloping isopycnals in the thermocline (C; Fig. 2b) lie beneath the pycnostad and may be horizontally offset from A.

A sharp change in water type extends into the thermocline beneath the front. On the isopycnal 26.0 kg m⁻³, for example (Fig. 2b, d), a change of 0.025 in salinity (salinities are given on the Practical Salinity Scale, 1978) and 0.2 ml l⁻¹ occurs in salinity and oxygen content at a run distance of ~1,710 km, with higher oxygen concentrations and lower salinities on the cold, north side of the front. Changes can still be seen as deep as the 26.3 kg m⁻³ isopycnal, which lies between 170 and 250 m depth.

The temperature/salinity (T/S) relation in the warm water wedge (densities <25.7 kg m⁻³) is very variable, and reveals water masses below the mixed layer that were not subducted locally. For example, the high-salinity water marked G (Fig. 2c) has a T/S relation found nowhere else in the section. It also

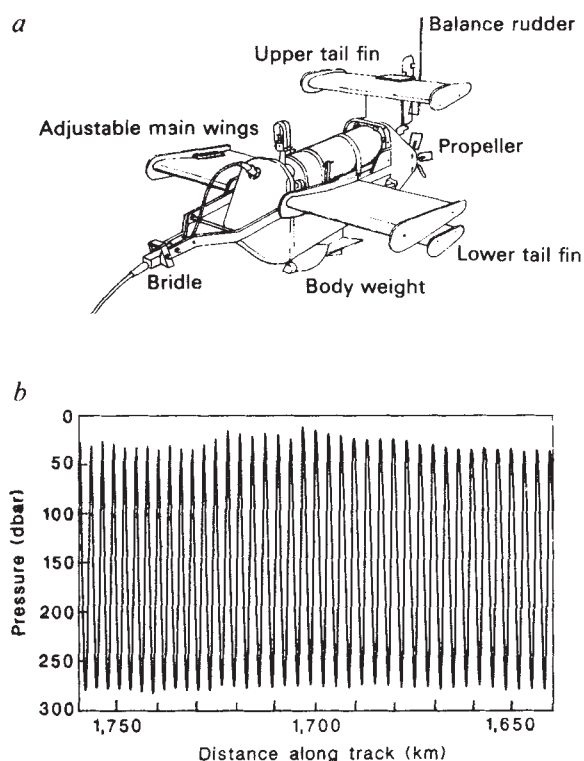


Fig. 1 a, The SeaSoar⁵, derived from the Canadian Batfish⁶, is a body with hydraulically powered, controllable wings. In FASINEX, it housed a Neil Brown CTD. b, Towed at 8 knots, the SeaSoar can dive and climb at an angle of about 1:5 to the horizontal, thus completing a sawtooth path to ≥ 300 m every 3 km. The shallow turn had to be made 10–30 m below the surface to avoid fouling by sargassum.

has weaker stratification than water of similar density nearby. On occasion, such regions of anomalous T/S relation are very weakly stratified (H, I; Fig. 3b). Comparison of H with the pycnostad B on a section 50 km further west suggests that these interior pycnostads contain water subducted from the mixed layer upstream.

Maximum velocities in the frontal jet (D; Figs 3, 4c) lie immediately adjacent to the surface density outcrop A on the low-density side. Mean vertical geostrophic shears between 50 and 250 dbar are as high as $2.5 \times 10^{-3} \text{ s}^{-1}$ (velocity difference of 50 cm s⁻¹). True current shears observed with an acoustic Doppler profiler on the same ship (L. Regier, personal communication) were of similar magnitude, perhaps 50% larger⁹. Comparison of true and geostrophic currents will form an important part of future analysis. Contoured horizontal shears on both sides of the frontal jet are as large as $6 \times 10^{-5} \text{ s}^{-1}$ ($6 \text{ cm s}^{-1} \text{ km}^{-1}$), comparable with the Coriolis parameter f , indicating that barotropic instability is probable^{4,10}. It is likely that true shears are larger, but have been smoothed by the gridding procedure.

The horizontal offset between mixed-layer (A) and thermocline (C) isopycnal slopes can broaden or bifurcate the main jet, D (Fig. 3a). The strongest jets occur when A lies above B (Fig. 3b). Additional regions of eastward flow (E; Figs 3, 4c) are found 30–40 km south of the main eastward jet, D. The cause of weaker (or possibly reversed) flow between D and E is the reversal of isopycnal slopes (F; Figs 2b, 3) just south of the pycnostad, B. This in turn suggests vertical circulation, with downwelling in the pycnostad and upwelling just south of it.

The map of temperature at 50 dbar (Fig. 4a) is similar to the surface temperature maps derived from infrared satellite data or aircraft surveys. A strong, linear east-west-oriented front is indicated.

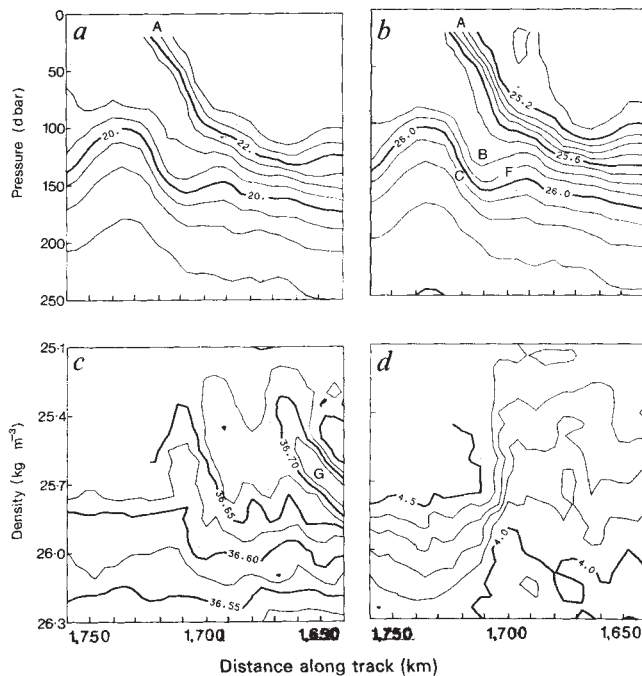


Fig. 2 Frontal features, shown in a north-south section (Leg 4 in Fig. 4a) of: *a*, potential temperature ($^{\circ}\text{C}$); *b*, density (kg m^{-3}); *c*, salinity and *d*, oxygen concentration (ml l^{-1}). Salinity and oxygen concentration are contoured with density as the vertical coordinate, to reveal changes in water-mass structure along density surfaces. Before contouring, the along-track (Fig. 1*b*), 1-s-averaged data are reduced to values on a rectangular grid with points 4 km and 10 dbar or 0.05 kg m^{-3} apart. Each grid point is an average of all 1-s values within $\pm 5 \text{ km}$ or $\pm 0.025 \text{ kg m}^{-3}$ of the point. This 10-km horizontal running mean removes internal waves (except possibly tides and inertial periods). A, frontal outcrop; B, pycnostad; C, steepest isopycnal slope in thermocline; F, reversal of isopycnal slopes; G, high-salinity water.

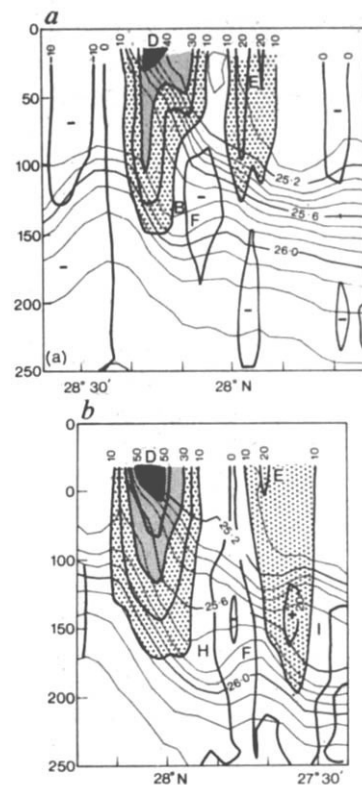


Fig. 3 Contours of eastward geostrophic velocity relative to a 250 dbar reference level (bold), superimposed on the density contours (see Fig. 2*c*) for: *a*, the section shown in Fig. 2; *b*, a section 50 km further east (Leg 1 in Fig. 4). The velocities are calculated from adjacent columns of the gridded density data (separated by 4 km; see Fig. 2 legend), without further smoothing. B, Pycnostad; D, main frontal jet; E, other regions of eastward flow; F, reversal of isopycnal slopes; H, I, weakly stratified regions of anomalous T/S relation.

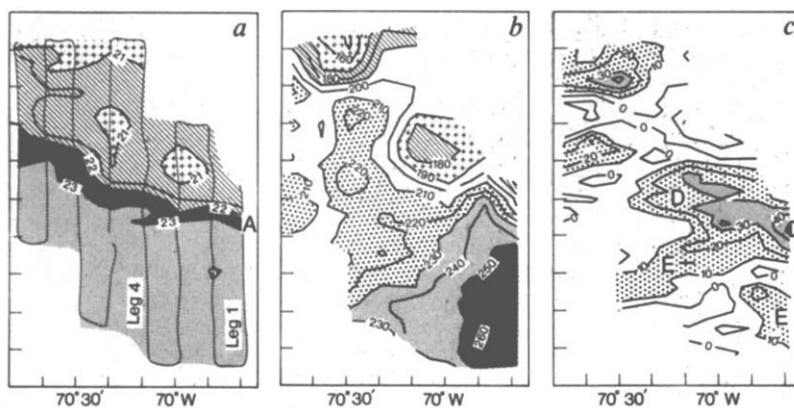


Fig. 4 Maps of mixed-layer temperature ($^{\circ}\text{C}$) at a depth of 50 dbar (*a*), the depth (dbar) of the 26.3 kg m^{-3} isopycnal (*b*), and eastward geostrophic velocities (cm s^{-1}) at 50 dbar relative to the 250 dbar level (*c*), spanning the entire survey ($120 \text{ km} \times 160 \text{ km}$) of 8 north-south legs, separated by $\sim 16 \text{ km}$. While the near-surface temperature field (*a*) appears linear and two-dimensional, an underlying eddy distorts the isopycnal depths (*b*), leading to a complex three-dimensional velocity field (*c*), and interrupting the surface jet, D. A, frontal outcrop; E, regions of eastward flow south of the main jet (D).

The complex features described above become apparent only when the pressure variations on an isopycnal surface in the thermocline are contoured (Fig. 4*b*). Isopycnal slopes strengthen from west to east, and a clockwise swirling eddy distorts the front in the middle of the survey area (see the 210 dbar contour).

Some of the features described here have already been simulated by models. The sharp change in water types is a consequence of confluence, which sharpens a front and tends to form a pycnostad beneath the frontal jet¹¹. Modulation of the spacing between isopycnals and vertical circulation are discussed by Bleck *et al.*¹². Fromtogenesis driven by Ekman conver-

gence is considered by Roden and Paskausky¹³. It is clear from Fig. 4*b,c* that three-dimensional models such as that being developed by Onken¹⁴⁻¹⁵ are necessary to describe most of the other observed features.

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Oceanographic implications of non-newtonian properties found in phytoplankton cultures

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Bulk rheological properties of sea water sometimes change in phytoplankton blooms¹⁻⁷, when the water has been described as viscous¹, slimey^{2,3}, ropery^{2,4} or like egg white⁴. Fishing nets have been clogged^{2,5,7} and broken², and bubbles⁶ or solids^{2,4,5} have been trapped. Turbulent viscosity, however, can be reduced⁸. It has been suggested^{1,2,6} that phytoplankton mucus may kill marine animals by 'clogging' their gills. Yet oceanographers consider the sea to be newtonian, that is without rigidity and with dynamic viscosity, η , independent of shear rate, γ . I show here that out of eight phytoplankton cultures investigated, η increased with decreasing γ in three, and the highest value of η , found at $\gamma = 0.017 \text{ s}^{-1}$, was ~ 400 times that found at unstated but probably high (10^2 – $5 \times 10^3 \text{ s}^{-1}$) values of γ by Miyaki and Koizumi⁹. Finite values of dynamic rigidity, G'' , were also observed. The characteristic length, L , and time, t , of Kolmogorov eddies¹⁰ are extremely sensitive to simulated γ -dependent η , and as the scales of many oceanic processes depend on L and t , the lack of rheometrical data at ambient γ could be detrimental to the modelling of these processes.

Table 1 lists the cultures used. They were grown in a light: dark regime of 12 h: 12 h at 18 °C at Nice. During transport the day before measurement, the temperature of the cultures dropped to ~ 8 °C for several hours, and in some cultures (A, B, E and *Noctiluca* in H) the flagellates sank to the bottoms of their culture vessels. The temperature was allowed to rise to ~ 20 °C for ~ 14 h before measurement, and in all cases the flagellates resumed swimming.

Measurements were made using a Low Shear 30 Sinus rheometer (Contraves AG, Zürich), fitted with a bob, 1.1 cm in diameter, suspended in a cup, 1.2 cm in diameter. Before adding fresh samples, the cup and the bob, both of stainless steel, were rinsed in acetone, then in ethanol, and blow-dried. The instrument was used mostly in constant- γ mode. γ was increased in steps, stress, τ , being read as soon as it had stabilized. In one run, the instrument was used in Sinus mode, in which shear, Γ , is varied sinusoidally from -0.12 to $+0.12$. This results in γ also varying sinusoidally, 90° out of phase with Γ , and with amplitude dependent on the frequency of oscillation. Using this method, the effects of elasticity may be distinguished from those of viscosity.

In cultures A, B, C, D and E, η was not detectably different from that of sea water, $\sim 1.0 \text{ mPa s}$, over the measured range of $0.15 < \gamma < 129 \text{ s}^{-1}$. Cultures F, G and H, which showed high values of η at $\gamma = 0.15 \text{ s}^{-1}$, were investigated over the range $0.017 < \gamma < 129 \text{ s}^{-1}$.

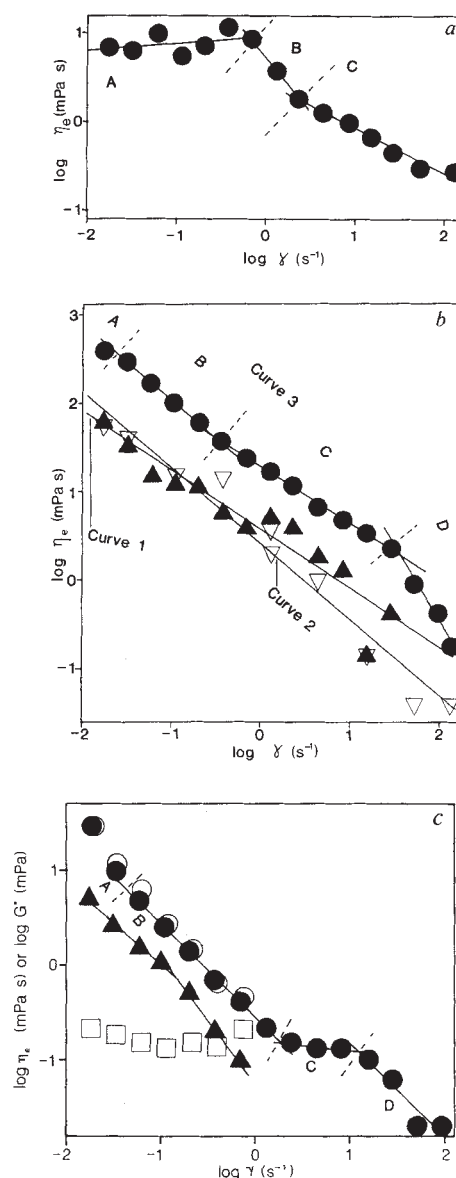


Fig. 1 Log-log plots of excess viscosity, η_e and dynamic rigidity, G'' versus shear rate, γ . p is given for some parts of the curves, with significance limits at probability > 0.9 for curves 1 and 2 in b; otherwise, probability > 0.95 . η_e was determined in constant- γ mode except where otherwise stated. a, Culture F: $p = 0.08 \pm 0.11$ for part A, -0.51 ± 0.05 for part C. b, Culture G. Curve 1 ($\blacktriangle$), first measurement of less concentrated sample, $p = -0.62 \pm 0.12$; curve 2 ($\triangle$), second measurement of less concentrated sample, $p = -0.86 \pm 0.14$; curve 3 ($\bullet$), sampled more concentrated in settled material, $p = -0.84 \pm 0.03$ for part B, -0.67 ± 0.03 for part C, -1.34 ± 0.08 for part D. c, Culture H. Curve 1 ($\bullet$), η_e measured at constant γ ; $p = -0.98 \pm 0.02$ for part B, -0.96 ± 0.30 for part D; curve 2 ($\blacktriangle$), as curve 1 but after sample filtration; curve 3 ($\circ$), η_e determined in Sinus mode (see text); curve 4 ($\square$), G'' determined in Sinus mode.

In the sea, γ varies from 0.0003 s^{-1} (ref. 11) to ~ 35 – 250 s^{-1} (calculated back to r.m.s. γ , assuming that $\eta \approx 1 \text{ mPa s}$ (ref. 9), from values of viscous dissipation of energy per unit volume, ϵ , for tumbled waves¹²). The range used in these measurements thus corresponds to the higher part of the natural oceanic range.

Excess viscosity, η_e , is here defined as $\eta - 1.0 \text{ mPa s}$. Figure 1a shows η_e for culture F plotted against γ . The culture behaves as a fluid ($p = 0$, where $\eta_e = k\gamma^p$) in part A of the curve, and shows behaviour compatible with that of a power-law fluid (p remains constant over a range of γ (ref. 13)) in part C. Micro-

Table 1 Phytoplankton cultures used

Culture	Species	Cell concentration (mm ⁻³)	Cell C* (g m ⁻³)	Relative volume†	Culture origin
A	<i>Prorocentrum micans</i>	19.3	21.1	1.43×10^{-4}	Indiana University, Strain LB1003 ³⁵
B	<i>Protoperdinium trochoideum</i>	51.2	29.8	1.97×10^{-4}	Indiana University Strain LB1017 ³⁵
C	<i>Dunaliella</i> sp.	1,350	29.0	2.77×10^{-4}	Isolated from Banyuls-sur-Mer; gift from CERBOM, Nice
D	<i>Amphidinium</i> sp. 1	569	54.4	3.18×10^{-4}	Indiana University, Strain LB1002 ³⁵
E	<i>Gonyaulax</i> ? sp.	23.5	84.9	6.42×10^{-4}	Isolated 18 October 1977 by J.-M. Pincemin from sample from Red Sea (J. Jaubert)
F	<i>Amphidinium</i> ? sp. 2	171	116.5	7.72×10^{-4}	Isolated from the surface of a sea anemone, <i>Aiptasia diasphora</i> , which had been collected from the Red Sea, and kept in artificial sea water at Nice
G	<i>Dunaliella marina</i>	83.8	3.1	1.72×10^{-5}	Gift from Station zoologique, Villefranche-sur-Mer
H	<i>Noctiluca scintillans</i> and <i>Dunaliella marina</i>	0.012 167	15.5 2.8	1.67×10^{-4} 1.50×10^{-5}	North Sea. Gift from G. Uhlig (Helgoland), December 1983 Same as culture G, but the cells were smaller than in culture G

Nomenclature is based on Parke and Dixon³³, where author citations for the species may be found.

* Cell organic carbon, calculated from measured cell volume by Strathmann's³⁴ method.

† Relative volume = volume of cells/volume of culture.

scopic examination of this culture revealed many transparent flakes <1–2 μm thick. Filtration through a 3- μm membrane filter gave a clear liquid in which η differed only slightly from 1.0 mPa s over a range of γ from 0.017 to 129 s⁻¹.

Material taken from near the bottom of culture G showed a power-law behaviour (Fig. 1b, curve 1). When the measurements were repeated after the sample had rested for 300 s (Fig. 1b, curve 2), the curve was significantly steeper (probability > 0.9), with η_e smaller at higher values of γ , indicating possible thixotropy. Curves 2 and 3 were fairly noisy, perhaps due to 'lumpiness', defined here as rheological patchiness.

A sample from the same culture but richer in sedimented material (Fig. 1b, curve 3) gave higher η_e values. The average slope was intermediate between those of curves 1 and 2, but the higher signal-to-noise ratio in this more concentrated sample allowed three zones, B, C and D, each of slightly different slope (probability > 0.95) to be distinguished.

Gentle filtration of this material through an 8- μm membrane filter gave a clear liquid in which, when γ was <1 s⁻¹ and steady, τ fluctuated widely about low values, giving values of η mostly <1.0 mPa s (not shown). This fluctuation may have been due to time-dependent effects, lumpiness or patchy adsorption of organic matter onto the cup or bob. Ionic dispersal of dissolved material may result in η for a solution being less than that for its pure solvent¹⁴, but I have found no mention of this effect in non-newtonian fluids.

Defining the excess stress as $\tau_e = \eta_e \gamma$, culture H (Fig. 1c, curve 1) showed two areas of constant τ_e , parts B and D, separated by an area of apparently constant η . After filtration through an 8- μm membrane filter, η_e (Fig. 1c, curve 2) decreased, for corresponding values of γ , by factors of 4–8. This culture, which had a *Dunaliella* concentration similar to that of culture G, showed little or nothing of culture G's relationship between η_e and γ . The material responsible for culture H's η_e/γ relationship is thus likely to have originated from *Noctiluca*, a heterotroph, which may have removed or changed polymers secreted by *Dunaliella*.

Using the same sample of culture H as that used to give curve 1 in Fig. 1c, curves of Γ against τ were obtained in Sinus mode.

η and G'' were calculated from measurements of these curves (A. G. Contraves, unpublished data). This η_e/γ relationship (Fig. 1c, curve 3) was fairly similar to that obtained using constant- γ steps (curve 1). G'' (curve 4) remained constant at 0.1–0.2 mPa for $0.017 < \gamma < 0.72 \text{ s}^{-1}$. Measurement at higher shear rates was precluded by inertial effects in the rheometer.

The relative volume of the plankton (Table 1) was far too low to affect measurably the bulk viscosity of the cultures¹⁵, and the thickening behaviour of many algal polysaccharides suggests that such materials caused the non-newtonian properties. Combined with analyses of excreted organic matter, rheological 'fingerprints' of algal and bacterial cultures might be useful taxonomically.

In constant, uniform conditions, turbulent energy cascades, at a rate ϵ , from the wavelength of energy input to progressively smaller wavelengths. The Kolmogorov hypothesis¹⁰ predicts that γ should peak, and the cascade be truncated, at the Kolmogorov characteristic length, $L = (\nu^3/\epsilon)^{0.25}$, where ν is the kinematic viscosity η/ρ , and ρ is the density of sea water, $\sim 10^3 \text{ kg m}^{-3}$. However, the wavelength at which γ peaks, while perhaps variable, has been found experimentally to lie nearer 50 L (ref. 16). Changes in γ -related η could have produced some of these discrepancies.

For some values of p characteristic of the phytoplankton cultures, Fig. 2a, b shows the calculated effect on L and t of imposing uniformly on the water a non-rigid substance (that is, one for which $G'' = 0$) of arbitrary excess viscosity, $\eta_e = 1.0 \gamma^p$. Phytoplankton exudates, which can reduce turbulent drag⁸ by elastic effects¹⁷, might modify the shear spectrum, and hence both L and t . Rheological heterogeneity (lumpiness) or departure from isotropy, however, would further modify the shear spectrum.

Mitchell *et al.*¹⁸, assuming that $\eta \approx 1 \text{ mPa s}$, have argued that when $\epsilon \leq 10^{-11} \text{ m}^2 \text{ s}^{-3}$, the characteristic timescale of Kolmogorov eddies, t , would be sufficiently long ($\geq 320 \text{ s}$) to allow unprotected microzones of increased metabolite concentration (MIMCs) to develop around plankters. There is evidence of MIMC protection by thick mucus^{18–20}. Yet it is well mixed^{19–21} ($\epsilon \approx 10^{-7}–10^{-6} \text{ m}^2 \text{ s}^{-3}$) or upwelling¹⁹ waters which tend to be

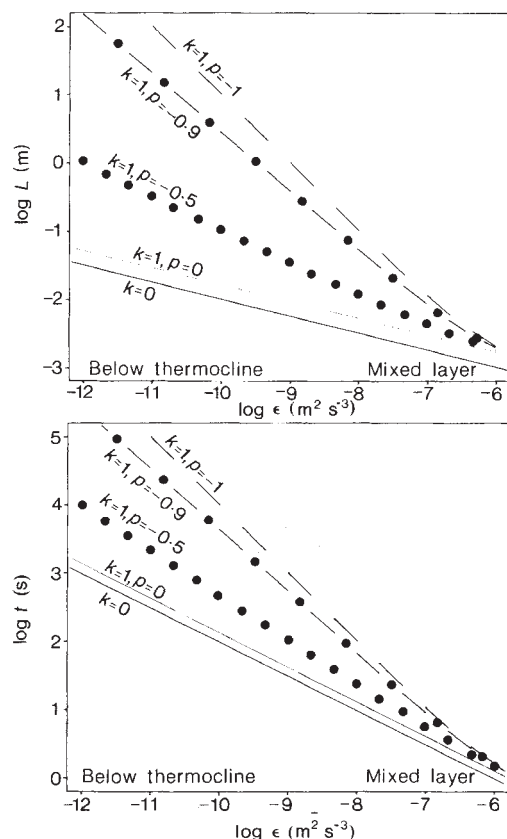


Fig. 2 Characteristics of Kolmogorov eddies calculated for water with an imposed substance of $\eta_e = k\gamma^p$ (mPa s). The dynamic viscosity of the water, η_w , is constant at 1.0 mPa s. Values were calculated by iteration to constancy, assuming that $\gamma = 1/t$. If γ is derived from r.m.s. $\gamma = [\epsilon/7.5(\eta\rho)]^{0.5}$ (ref. 12) (not shown), smaller values of γ are obtained, so that when $p < 0$, L and t are even greater. *a*, log (Kolmogorov characteristic length, L) versus log (rate of viscous energy dissipation, ϵ). *b*, log (Kolmogorov characteristic time, t) versus log ϵ .

characterized by thickly mucilaginous phytoplankton such as *Phaeocystis pouchetii*²⁰ or certain *Thalassiosira* species²¹. *A priori*, mucous protection of MIMCs might seem to be most cost-effective, and therefore likely to be most widespread, in waters of low to intermediate values of ϵ (10^{-11} – 10^{-7} m² s⁻³). In such waters, however, MIMCs may be effectively protected by mucus in less noticeable amounts than in well mixed water.

Because the size and lifetime of planktonic MIMCs are positively related to L and t (ref. 18), they depend on η , as well as on G'' . If, as suggested¹⁸, MIMCs are important in zones of low ϵ , then η and G'' may determine the structure and dynamics of plankton populations.

Certain aggregates are held together by diatom chains or setae²², whose functions and rheological properties at length scales $\gg 1$ mm may be analogous to those of aggregates held together by polymer molecules. The faster the aggregates sink, the greater will be their tendency to break into smaller particles, which sink more slowly. As suggested for sewage flocculates in the sea²³, the sinking rate of marine snow may be governed by its resistance to break-up. Both mucus and setae may enhance MIMC formation within the aggregate.

Marine phytoplankton generally excrete 5% (ref. 24) to 20% (ref. 25) of the carbon they fix, but more may be released under stress²⁴. Concentrations of dissolved organic carbon (DOC) in shallow and deep seas range from 0.4 to 7 g m⁻³, being generally highest and most variable in the photic layer^{26–28}. While the proportion of DOC in molecules of very high ($>10^5$) molecular

weight (MW) has been found to be ~15% in coastal waters²⁷, in deep water it may reach 47% (ref. 28). As this deep-water DOC has an overall radiocarbon age of ~3,500 yr (ref. 29), this indicates that a pool of refractory, very high-MW material contributes a large proportion of deep-water DOC. Of peptides and polysaccharides excreted by phytoplankton^{20,24} and bacteria³⁰, the low-MW (<900) fraction is used preferentially by heterotrophs^{28,30}. This may result in a distinct pool of young DOC of high (900–10⁵) and very high MW, abundant in zones of active primary production, especially when the plankton there is stressed. Such may be the case in many red tides, which could account for their sometimes grossly unusual rheology.

Levels of DOC are generally only 2–5 times higher in surface waters than in deep waters^{27,28}, and values of γ (calculated back from reported values of ϵ varying by more than five orders of magnitude) are generally lowest below the thermocline^{16,31}. Thus, the values of p found in culture (0 to -1.3) suggest that the highest values of η in the oceans are as likely to be associated with low-shear deep water as with high-DOC productive zones.

The relationship between air-sea diffusion and ϵ depends on both η and L (ref. 12), and thus also on G'' . Cases of extreme *in situ* variation in η and G'' have been cited above. Less extreme variation, however, could account for some of the unexplained difficulties in fitting data from different geographical areas to models, not only of both gas and heat flux³², but also of energy transfer from wind to sea turbulence³¹. Rheological study of the sea is thus required at ambient shear rates and at length scales down to those of the smallest-scale turbulence.

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Stomata and sterome in early land plants

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Recognition of pioneering land plants in the fossil record is highly contentious. Because vascular plants possess numerous structural modifications which maintain an internally hydrated environment, attempts to demonstrate the vascular status of megafossils have traditionally dominated research, although more recently evidence from microfossils suggests that Ordovician and Silurian land vegetation may have included plants with some attributes of bryophytes^{1,2} and thallophytes^{3,4}. Silurian megafossils are preserved as impressions or coalified compressions⁵, in which anatomy is rarely preserved. The claim to land plant status of Ludlow and Pridoli examples of the presumed rhyniophytes *Cooksonia* and *Salopella* is based on their axial architecture, and hence presumed erect growth habit, and on their *in situ* spores with sporopollenin. Here we report on exceptionally preserved coalified fossils from the basal Devonian of Shropshire which show that *Cooksonia* also possessed stomata and thick walled supporting tissues although evidence for vascular tissue still eludes us.

To the naked eye the axial fossils, preserved in a grey siltstone dated as early Gedinnian by palynomorphs, are unexceptional with no obvious sporangia. After dissolving the matrix in hydrofluoric acid, the residue contained abundant opaque dark fragments a few millimetres long. These were mounted on stubs for examination uncoated in an environmental chamber attached to a ISI 60A scanning electron microscope (SEM). Some were then remounted on stubs and coated for more detailed examination using a Cambridge 180 SEM. The remainder were cleared with Schultz's solution or were embedded and sectioned. Sporangia and some axes responded differently to oxidation. Certain axes remained opaque for many hours and although flattened (Fig. 1a) show a lack of compression in the peripheral layers of cells, with homogenization of their walls. Such features are characteristic of charcoal⁶ but more detailed analyses, including reflectance studies, are required.

The macerate is distinguished by the abundance and diversity of terminal sporangia: globose, discoidal and fusiform sporangia are present, almost all containing spores. Here we concentrate on those sporangia that resemble *Cooksonia pertoni*. Detailed studies based on a large number of coalified compressions from the type locality⁷ have yielded information on spores and sporangial wall organisation and enable recognition of the species when the sporangia are compressed in different planes. Figure 2 shows in side view an intact sporangium from the new locality, its surface partly covered by a cuticle. In a further specimen, details of the wall are clearly visible where the left hand part is split open, revealing a single layer of uniform brick shaped cells with their long axis at right angles to the surface (Fig. 3a). A dehiscence line has not been observed. Figure 3b illustrates a gaping stoma found on an expanded axis just below a sporangium. It is not possible to observe the number of guard cells present because of the cuticular covering which also obscures the outlines of epidermal cells. At the fractured end of the same axis these cells are revealed as isodiametric in cross section and elongate with tapering ends (Fig. 1b). Together with one or two hypodermal layers, they constitute a peripheral thick-walled supporting tissue; a sterome. Trilete spores (Fig. 3c) with ornamented distal surfaces removed from a further sporangium are referable to a variety of the dispersed taxon *Streelispora newportensis*. These spores, like all those known from Downton specimens of *C. pertoni*, have equatorial crassi-

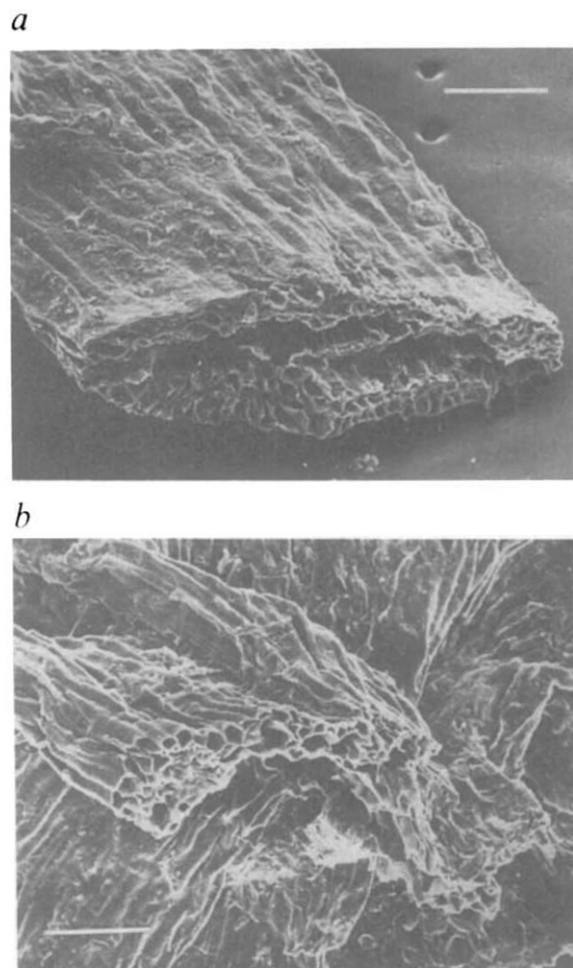


Fig. 1 Scanning electron micrographs of fractured ends of axes showing peripheral supporting tissues. a, unidentified sterile axis (UCC. S/HD 1A); b, axis below *Cooksonia* sporangium with stoma (UCC. S/HD 1D). Scale bars, 100 μ m.

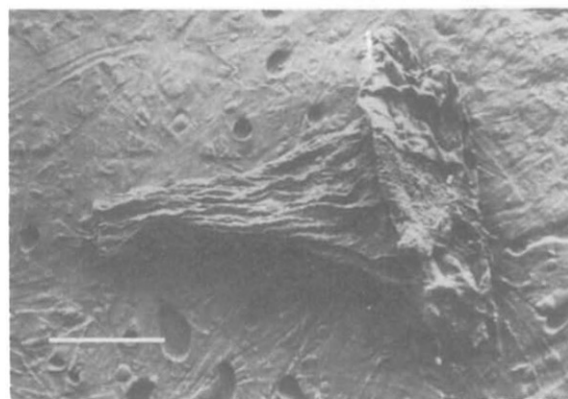


Fig. 2 Scanning electron micrograph of intact *Cooksonia* sporangium (UCC. S/HD 1F). Scale bar, 300 μ m.

tudes whereas the Ditton specimens have a distal conate sculpture. In the dispersed state the taxon *S. newportensis* is highly variable, in contrast to that of the *in situ* spores which (at least distally) are more or less the same.

On the basis of overall shape, wall characteristics and the relationship between sporangium and its subtending axis, these specimens would unhesitatingly be assigned to *C. pertoni*. The absence of branching in fertile specimens is probably

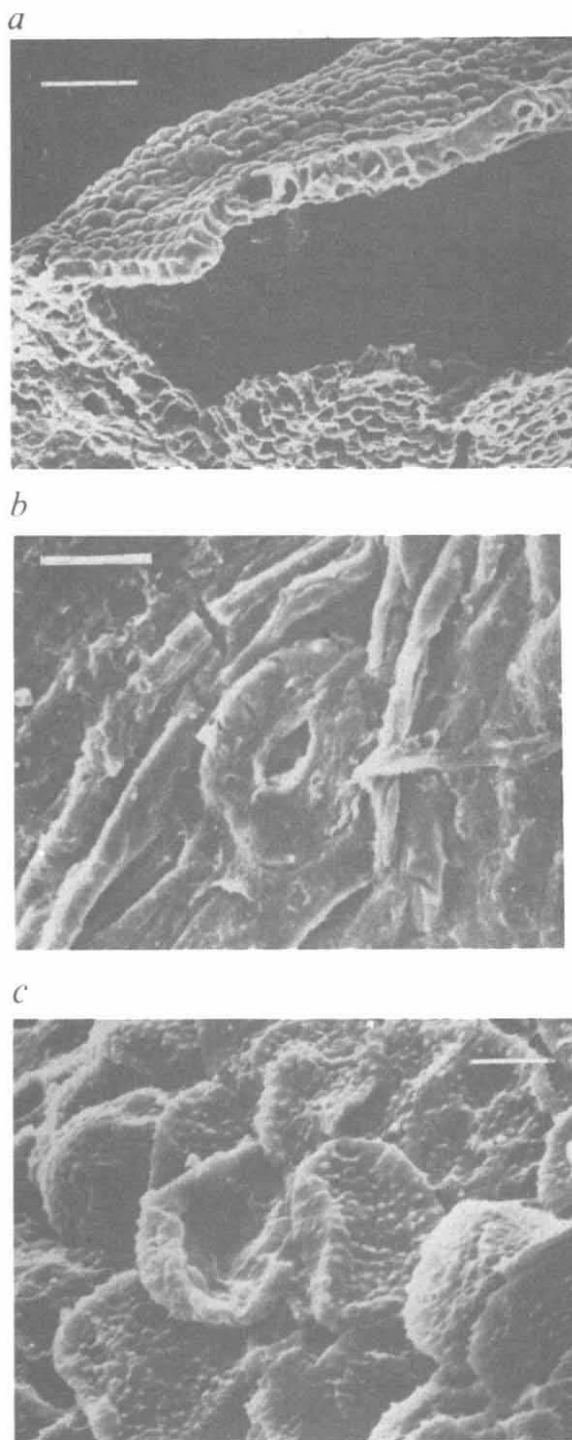


Fig. 3 Scanning electron micrographs showing cellular preservation in *Cooksonia*. *a*, Detail of wall of sporangium (UCC. S/HD 1B); scale bar, 60 µm. *b*, Stoma in subtending axis; scale bar, 20 µm; arrow indicates indentation (UCC. S/HD 1D); *c*, *In situ* spores (UCC. S/HD 1E); scale bar, 20 µm.

symptomatic of the preservation. Although we cannot be certain that the sporangia terminated isotomously branching axes, the lack of marked overtopping in the associated assemblage of sterile axes suggests that the plants possessed a growth habit typical of *Cooksonia*. A notable difference from *C. pertoni* is the presence of ornamented spores. We have already encountered a parallel situation at the type locality. Lang⁷ recorded smooth walled spores in *C. pertoni* but we have also recovered orna-

mented spores, *Synorisporites verrucatus* from morphologically similar sporangia. As it seems unlikely that these three forms represent different developmental stages of the same spore, we conclude that three plants which are indistinguishable morphologically, and are anatomically similar as far as is known, possess spores of identical structure but variable or nonexistent sculpture. The verrucate to murinate sculpture of the Downton specimens is succeeded by an apiculate one in the Ditton group. This is a familiar pattern in Silurian and early Devonian dispersed spore sequences where the early sculptured representatives of a particular structural type tend to be murinate or verrucate, with apiculate spores appearing later. The implications for the identification and naming of such plants will be explored more fully elsewhere.

Here we show the presence of stomata and a sterome in *Cooksonia* for the first time. Since unequivocal evidence for its vascular status^{7,8} is still lacking, the genus remains better described as rhyniophytoid⁹. Further collecting should however provide sufficient material to permit the destructive techniques necessary to demonstrate *in situ* tracheids.

The assemblage described here is early Devonian. Is it feasible to infer that earlier cooksonias also possessed stomata and sterome? The answer has particular relevance to their status as land plants, and to their habitats. In 1984 Gray¹ suggested that the Northern Hemisphere rhyniopsids so closely resembled the better known Rhynie Chert plants that their ecology was probably similar, that 'the weakly developed conducting system only a few tracheids thick (the presence or absence of phloem is equivocal because of preservation), the thin epidermal cuticle without stomata in the Silurian members of the group, and the thin-walled tissues could be used to argue that bryophytes confined to water-saturated situations or bogs are more reasonable ecological equivalents than vascular plants'. She concluded with the possibility 'that the rhyniophytes were amphibious plants that developed some adaptations of the vascular plants but never migrated beyond an environment similar to that in which *Rhynia* is found'.

Considering first the occurrence and significance of the sterome, film pulls taken from Ludlow and Pridoli axes from South Wales frequently show longitudinally orientated thick-walled cells¹⁰, presumably in peripheral regions, although these have not yet been demonstrated in fertile material, nor in the earlier Irish Wenlock *Cooksonia* assemblage¹¹. Preservation of such plants in clastic sediments may have depended on the presence of such decay-retarding tissue. Its function in support would be particularly important for plants in which water conducting tissues were absent or weakly developed¹² and which grew in environments where water was not always readily available. Later Lower Devonian plants preserved in Old Red Sandstone facies almost always possessed thick walled peripheral tissues, interpreted as an adaptation to the seasonal climate of the Old Red Continent¹³ in that they would have maintained the erect habit of the plant in times of drought. In contrast, the predominance of thin walled tissues in Rhynie Chert plants suggests reliance on a hydrostatic skeleton for support. We agree with Gray that, in earlier Silurian times, there may well have been similar swamps colonised by plants with *Rhynia* type organisation (and sadly of low fossilization potential) but that it is equally possible the plants lived in drier and less hospitable areas too. Beerbower¹⁴ points out that before the development of extensive land vegetation, soils would have been of low nutrient status and moisture-holding capacity, with major fluctuations of temperature and physical instability. We envisage that small plants similar to *Cooksonia* with well developed support tissues would have been able to tolerate such inimicable conditions.

There is some evidence for cuticle but none for stomata in pre-Devonian axial fossils from the northern hemisphere. Thus we can still only speculate on whether or not stomata were present in the earliest cooksonias or whether they were acquired

later, possibly originating as pores connecting an intercellular space system with the outside air, and then evolving the ability to regulate gaseous exchange¹⁵. *Zosterophyllum* stomata are often cited as moss-like because they appear to possess a single guard cell. The presence of a small indentation of T shaped thickening at the stomatal poles and occasional traces of a line leading to the pore are however suggestive of the two guard cells of a conventional stoma¹⁶. Such indentations are also seen in stomata on our new material which is approximately the same age as *Z. myretonianum*. Connection with an intercellular space system will be difficult to demonstrate; presumably the sterome was interrupted below each stoma, but the latter are so infrequent that chance fracture is unlikely to include them.

An alternative even more speculative approach relating to occurrences of stomata in Silurian plants of *Cooksonia*-type organisation involves their position in the phylogeny of higher green plants. A strongly favoured hypothesis postulates that characters including stomata common to bryophytes and vascular plants support a single migratory event by some group of charophycean algae^{3,17,18}. Should the archegoniate descendants of this ancestral group have evolved stomata and cuticle, then these features would also have been present in the earliest rhyniopsids and rhyniophytoids. There are of course other scenarios but it is not our intention to speculate on the phylogeny of the Bryophyta and Tracheophyta. The current uncertainty reflects the magnitude of the imponderables and the sheer frustration of research activity on early land plants. We need

more fossils. The quality of preservation in this new material renews optimism for investigations on coalified compressions.

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Two identified afferent neurones entrain a central locomotor rhythm generator

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Sensory feedback can modulate the intensity and timing of the central rhythms underlying locomotion and adjust them to compensate for natural or experimental perturbations^{1–4}. However, because of the complexity of the neural systems involved, the role of specific sense organs and the function of individual afferent neurones are poorly understood. The thoracic-coxal muscle receptor organ (TCMRO), a proprioceptor of the crayfish walking leg, has only two afferent neurones, the non-spiking S and T fibres. Their receptor potentials encode, respectively, the magnitude and velocity of receptor muscle stretch, which occurs during limb retraction. Rhythmically stretching the TCMRO entrains a central locomotor rhythm, produced by the thoracic ganglia, in which remotor and promotor motoneurons of the leg discharge in alternation. Intracellular stimulation of the S and T fibres can trigger promotor and remotor bursts, respectively. Here we propose a mechanism for proprioceptive entrainment in terms of the opposite feedback effects of these two afferents. The possible role of these effects in the feedback control of locomotion is also discussed.

We use an isolated preparation^{5–7} of the thoracic nerve cord of the crayfish (*Pacifastacus leniusculus*), with the TCMRO of the fourth walking leg left attached to its segmental ganglion (Fig. 1a). The TCMRO spans the articulation of the leg with the thorax and lies in parallel with the limb promotor (or

protractor) muscle⁸. *In situ* it is stretched by limb retraction (Fig. 1ai), as occurs during the stance phase (or power stroke) of forward walking. Stretch evokes graded depolarizing receptor potentials in two non-spiking afferent neurones, the S and T fibres^{6,9–11}. The receptor potential of the S fibre is primarily a static indicator of receptor length, while the T fibre is dynamically sensitive to the velocity of receptor stretch (Fig. 1b). These receptor potentials are conducted decrementally to the central nervous system (CNS) where, in quiescent preparations, they excite coxal promotor motoneurons (MNs) in a negative-feedback resistance reflex^{10–14}. Like the vertebrate stretch reflex, this would resist the imposed movement and may serve a postural role. In excitable preparations, however, the reflex can reverse in sign to produce a positive-feedback assistance reflex, so that remotor MNs are now excited and promotor MNs inhibited^{6,15,16}. In such preparations the S and T fibres exert opposite reflex effects; the T fibre excites remotor MNs and the S fibre excites promotor MNs^{6,16}. In both cases the antagonistic group of MNs is reciprocally inhibited.

The isolated ganglion often produces bouts of rhythmical activity in walking leg MNs. Promotor and remotor MNs discharge in strict alternation, while levator MNs are active during the promotor phase^{7,16}. Because levator discharge *in vivo* underlies lifting of the leg in its swing or return stroke, this pattern of coordination resembles that used during forward walking. The spontaneous rhythm, which is often irregular in frequency, is profoundly affected by afferent input. Periodic stretch and release of the TCMRO can entrain the rhythm, so that the alternating bursts now occur regularly and predictably (Fig. 2a). The remotor burst locks onto stretching and the promotor burst onto relaxation; that is, the relationship of motor output to TCMRO length change is similar to that occurring during forward walking in the intact animal.

The TCMRO governs the timing of the rhythm generator as well as providing reflex reinforcement to the MNs (Fig. 2). While the entrained rhythm bears an obvious resemblance to a series of assistance reflexes, the motor bursts are central in origin, as reciprocal bursting is present before and after the entraining stimulus (Fig. 2a), and motor bursts can escape from 1:1 entrainment, outlasting one stimulus cycle while their antag-

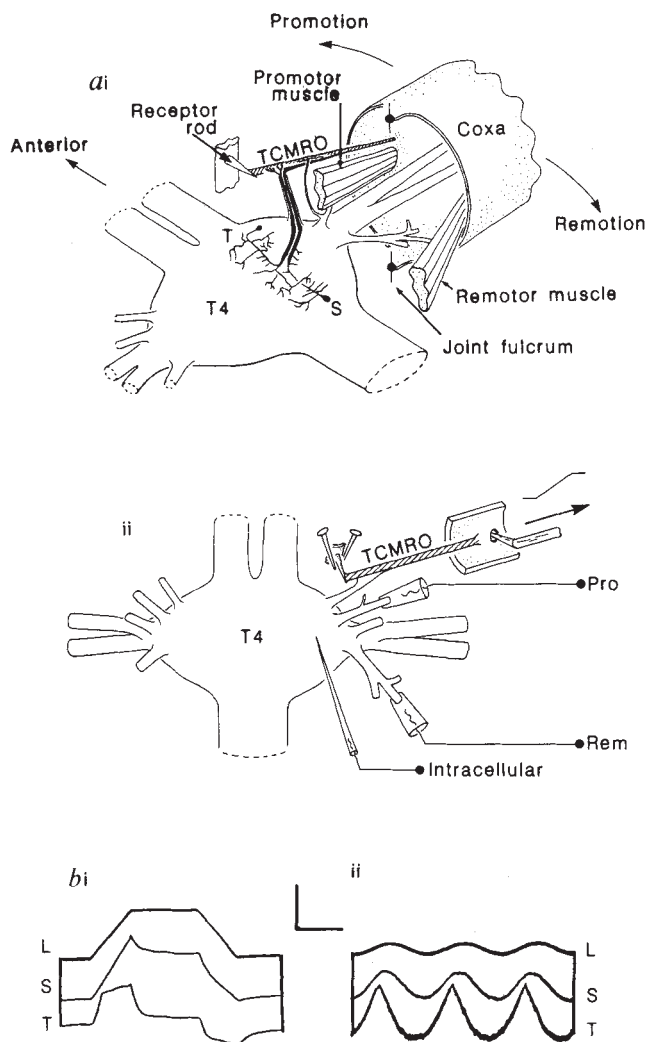


Fig. 1 The thoracic ganglion-TCMRO preparation and afferent receptor potentials. *a* i, Diagram showing the *in situ* arrangement of the TCMRO of the fourth thoracic ganglion (T4), which lies in parallel with the coxal promotor muscle and inserts beside the promotor tendon on the anterior rim of the coxa. Proximally the TCMRO originates in the thorax from a cuticular receptor rod arising from an endosternal pillar. Remotion of the coxa stretches the TCMRO. The TCMRO receives efferent innervation from a branch of the coxal promotor nerve, but this was always cut, so the experiments reported here represent open loop conditions. The S and T afferent neurones are represented semi-schematically from camera lucida drawings of Lucifer yellow stains^{7,28}. ii, The isolated ganglion-TCMRO preparation. The third, fourth and fifth thoracic ganglia were dissected out (only the fourth illustrated), together with the TCMRO of the right fourth walking leg, and pinned on Sylgard in oxygenated crayfish saline. The TCMRO was fixed proximally by its receptor rod and attached distally by a chip of coxal cuticle to an electromagnetic pulling device. Suction electrodes were used to record from the cut ends of the promotor and remotor nerves, and glass microelectrodes to record intracellularly from the neuropilar processes of the S and T fibres and leg motoneurons. Further details of the preparation are given in ref. 7. T4 is ~2 mm in diameter. *b*, S and T fibre receptor potentials recorded in the neuropile during stretching of the TCMRO. i, A ramp stretch-hold-release stimulus shows the length sensitivity of the S fibre and dynamic sensitivity of the T fibre; ii, sinusoidal stimuli show the phase advance (~50°) of the T-fibre response over the stimulus; note also that T has a phase advance over S. Upward deflection of the length monitor traces (L) indicates TCMRO stretch (as in subsequent figures). Scale bars: horizontal, 1 s (bi), 0.5 s (bii); vertical, 10 mV (20 mV for T in bi), 1 mm (bi), 2 mm (bii).

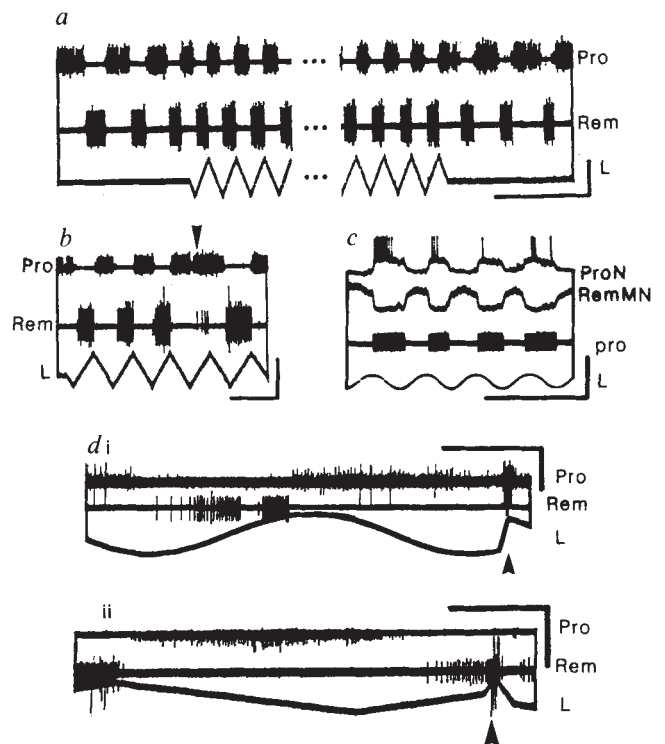


Fig. 2 Entrainment of the central rhythm by TCMRO stimulation. *a*, A spontaneous motor rhythm is entrained by repetitive stretch and release of the TCMRO for the duration of the stimulus (2.5 cycles omitted from middle). *b*, Occasionally one burst (here in promotor, arrow) extends over two stimulus cycles, while the antagonists (remotors) are silenced. *c*, Bursts of spikes and membrane potential oscillations, recorded intracellularly in two neurones during an entrained rhythm. These oscillations are similar in sharp onset and offset to those occurring in the spontaneous rhythm^{7,16}. *d*, Phase-dependent reflexes elicited by fast superimposed stretch during the entrained rhythm: i, during the promotor phase, stretch excites promotor motoneurons (arrow); ii, during the remotor phase, stretch further excites remotors (arrow). Abbreviations. Extracellular recordings: Pro, promotor muscle nerve; Rem, remotor muscle nerve; intracellular recordings: RemMN, remotor motoneurone; ProN, presumed motoneurone active in phase with promotor. Scale bars: horizontal, 10 s (*a-c*), 2 s (*d*); vertical, 1 mm (*a, b, di*), 0.7 mm and 10 mV (*c*), 0.5 mm (*dii*).

onists are silenced (Fig. 2*b*). Furthermore, the entrained bursts resemble those of the intrinsic rhythm in the sharp onset and offset of the underlying oscillations in MN membrane potential (Fig. 2*c*). If fast stretches of the TCMRO are superimposed on the more slowly entraining stimulus, a reflex response is evoked which depends on the phase of the entrained rhythm. Thus, a fast stretch applied during the promotor phase evokes additional reflex discharge in the promotor nerve (Fig. 2*di*), whereas during the remotor burst it causes reflex excitation of remotor MNs (Fig. 2*dii*). It seems unlikely that this result would be obtained if entrainment were due to a simple, graded assistance reflex. Instead, it suggests that the TCMRO directly influences the central rhythm generator which, in turn, modulates TCMRO-mediated reflexes according to the phase of the rhythm. As in the unentrained motor pattern, assistance reflexes are evoked during the remotor burst, but during promotor activity resistance reflexes occur^{5,6,16}.

Static and dynamic components of the sensory stimulation have opposite effects on the timing of the rhythm (Fig. 3). During spontaneous rhythm generation, rapid stretch of the TCMRO late in a remotor burst elicits a brief reflex discharge of remotor

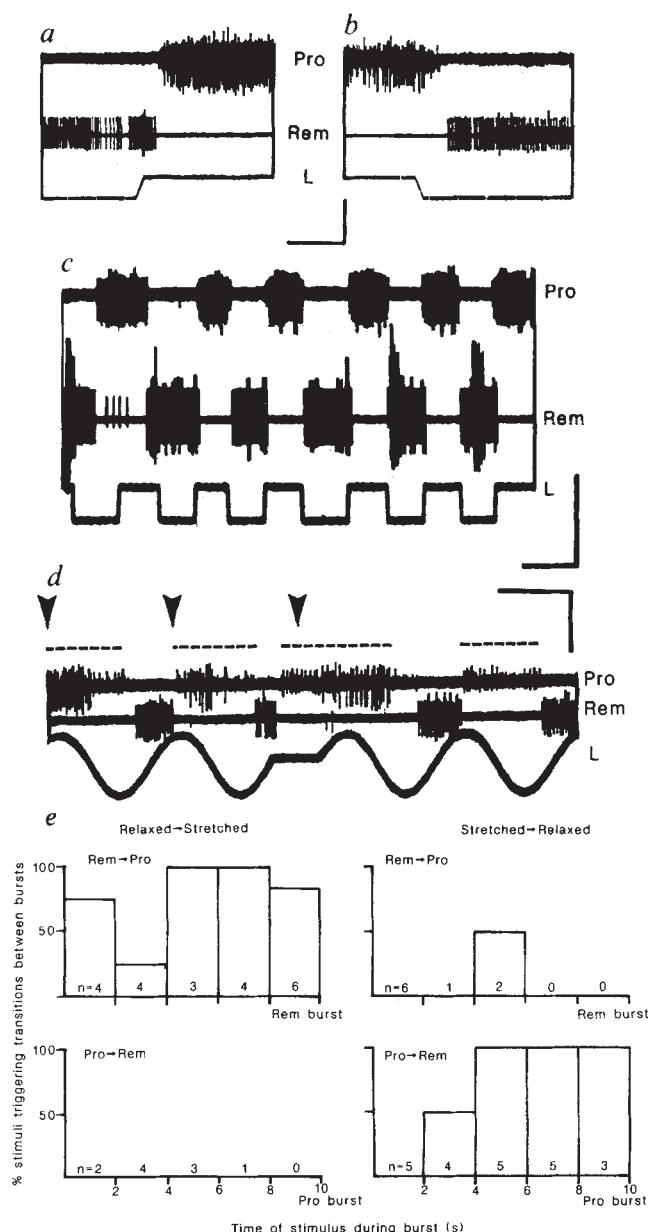


Fig. 3 Static (*a-c*) and dynamic (*d*) effects of TCMRO stimulation on the spontaneous motor rhythm. *a*, Rapid stretch of the TCMRO late in a remotor burst prematurely terminates remotor discharge (following a transient dynamic reflex excitation) and initiates promotor bursting. *b*, Conversely, rapid relaxation of the TCMRO late in a promotor burst does the reverse. *c*, Appropriately timed transitions between stretched and relaxed TCMRO lengths cause promotor bursts to be confined mainly to the stretched position. *d*, During entrainment by sinusoidal TCMRO stimulation, interrupting the stretching movement arrests the remotor burst and initiates the promotor burst (promotor bursts are marked by broken lines). The timing of the rhythm is therefore reset. Arrows indicate the predicted time of onset of promotor bursts. *e*, Quantitative analysis of the effects illustrated in *a-c*. Transitions from a relaxed to a stretched TCMRO length (left) trigger a switch from remotor to promotor bursting (top) but not the opposite switch (bottom). Transitions from a stretched to a relaxed length (right) trigger a promotor to remotor switch (bottom), but trigger the opposite switch in only one of nine tests (top). The ability of a stimulus to trigger a switch is apparently greater later in a burst, but stimuli can terminate a burst both before and after the time of its mean spontaneous termination. In a preceding bout of five cycles of rhythm in the absence of stimulation, the mean ($\pm$ s.d.) duration of remotor bursts was $5 (\pm 1.6)$ s, that of promotor bursts $5.9 (\pm 1.3)$ s. Scale bars: horizontal, 1 s (*a, b*), 10 s (*c*), 4 s (*d*); vertical, 1 mm (*a-d*).

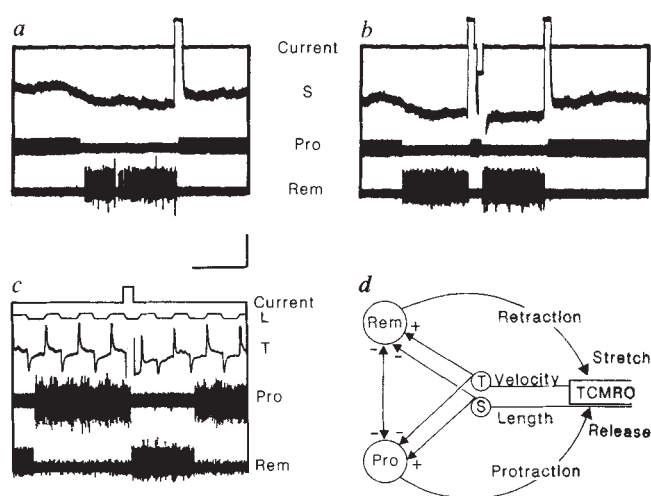


Fig. 4 *a-c*, Timing effects of stimulating individual afferents during spontaneous rhythmic activity. *a*, A brief depolarizing current pulse injected into S late in a remotor burst initiates promotor bursting. *b*, A brief hyperpolarizing pulse into S restarts a remotor burst that had been terminated by a preceding depolarizing pulse. A subsequent depolarization causes promotor bursting to resume. No stretch is applied to the TCMRO. Note in *a* and *b* the oscillation in S-fibre membrane potential in phase with the rhythm, due to central synaptic inputs (see ref. 7). *c*, A brief depolarizing pulse into T late in a promotor burst triggers the onset of the remotor burst. The TCMRO was stretched repetitively at a higher frequency, eliciting typical T-fibre receptor potentials. Note also that the onsets of the two promotor bursts (and offsets of remotor bursts) follow the negative dynamic responses of the T fibre. *d*, Schematic diagram to summarize the effects described in the present study. 'Rem' and 'Pro' represent the remotor and promotor motoneurons together with their associated burst-generating networks. See text for further details. Scale bars: horizontal, 2 s (*a-c*); vertical, 1 mV, 20 nA (*a, b*), 10 mV, 40 nA, 4 mm (*c*).

MNs, immediately followed by promotor bursting during the maintained stretch (Fig. 3*a,e*). The opposite stimulus late in a promotor burst triggers the start of remotor activity and terminates the promotor burst (Fig. 3*b,e*). Appropriately timed stimuli can therefore cause promotor bursts to be confined to the stretched position, while remotor bursts occur in the relaxed position (Fig. 3*c*). Hence, promotor bursting is excited by a static signal sensitive to stretch. Remotor bursting, by contrast, is excited by a dynamic signal, sensitive to stretching movement. For example, during entrainment by sinusoidal stimulation, interrupting the stretching phase prematurely terminates the remotor burst and initiates promotor activity, thereby resetting the timing of the rhythm and its phase relation to the stimulus (Fig. 3*d*).

These static and dynamic signals are carried by the S and the T fibre, respectively. Brief changes in the membrane potential of each afferent, induced by current injection, have opposite effects on burst timing, and can switch between the two phases of the motor output (Fig. 4*a-c*). Depolarization of the S fibre late in a remotor burst prematurely terminates it and triggers promotor activity (Fig. 4*a,b*). Hyperpolarizing pulses into the S fibre (Fig. 4*b*) or depolarizing pulses into the T fibre (Fig. 4*c*) effect the opposite switch.

Entrainment by the TCMRO (for example, Fig. 2*a*) can be understood in terms of the reciprocal effects of the S and T fibres on burst generation (summarized in Fig. 4*d*). Because of its dynamic sensitivity, the T fibre responds to stretch of the TCMRO with a phase advance over the S fibre (Fig. 1*bii*). Following the onset of stretching, therefore, the remotor burst is excited. At or near maximal TCMRO length, the velocity of stretch and therefore the dynamic response of the T fibre

decreases; the T fibre rapidly repolarizes but the static S fibre remains close to its maximum depolarized level. Now S-fibre effects prevail and excite promotor bursting. The S fibre probably supports the promotor phase throughout release as it gradually repolarizes. According to this scheme, the role of the T fibre is to initiate remotor activity and entrain it to stretch of the TCMRO, while the S fibre functions to switch from remotor to promotor activity when maximum length is reached.

This organization of feedback pathways will have important consequences for the control of forward walking in the intact animal, where the rhythmical stimulation of the TCMRO may affect both the timing and intensity of motor bursts. As the leg retracts during the stance phase, the dynamically sensitive T fibre will reinforce remotor bursting. Positive feedback provided by a dynamic sensory signal may be necessary to ensure that the motor output occurs in synergy with the movement it would normally produce¹⁷. When the limb reaches a fully retracted position, the S fibre will excite promotor bursting and may contribute to the transition from stance to swing. However, our results suggest that during entrainment this transition is not determined by an absolute value of position, signalled solely by the S fibre, but rather by the net interaction of static and dynamic feedback effects.

Whether this organization of feedback effects occurs in other systems is unknown. In most animals, however, feedback concerning limb or body position contains both static and dynamic components. Moreover, remarkable similarities of motor control in invertebrates and vertebrates are already emerging¹⁸. In both groups reflex pathways are phasically modulated by the CNS during locomotor activity^{5-7,16,19-21}, and may themselves profoundly affect the underlying motor rhythms^{3,4}. Behavioural studies have shown that the stepping movements of walking crustaceans²², insects²³ and cats^{3,17} can all be entrained to imposed rhythmical movement of the legs. In each case a retracted position of the leg-to-body joint is important in timing the transition from stance to swing²⁴⁻²⁶. A dynamic reinforcement of stance phase activity has also been reported¹⁷. In stick insects, stretching a leg proprioceptor that is extended during the stance phase of walking can dynamically excite stance phase MNs and, at a stretched length, initiate swing phase activity²⁷. Dynamic reinforcement of power stroke activity and triggering of the return stroke by a static signal may therefore be general phenomena. Our results show how these two types of feedback effect can be mediated, respectively, by the two identified afferents of a single muscle receptor organ, one sensitive to velocity and the other to position, during the generation of an endogenous motor rhythm. The crayfish preparation described here thus offers an opportunity to understand the feedback control of locomotion at the cellular level.

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Schistosoma mansoni shares a protective oligosaccharide epitope with freshwater and marine snails

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The expression of similar antigenic determinants by trematode parasites and their intermediate (invertebrate)¹⁻⁵ or definitive (vertebrate)⁶ hosts has been previously reported. Studies of experimental⁷⁻⁹ and human¹⁰ infection by the parasite *Schistosoma mansoni* have revealed the strong immunogenicity of a surface antigen with a relative molecular mass (M_r) 38,000 (38K). Here we provide evidence that the important protective epitope of the 38K molecule is expressed by the uninfected intermediate host of *S. mansoni*, *Biomphalaria glabrata* and is synthesized both by the mollusc and by the parasite throughout its life cycle, thus confirming our original hypothesis¹¹. Deglycosylation experiments indicate that the protective epitope is an oligosaccharide and in *B. glabrata*, is associated with a 90K component. Analysis of soluble extracts from different freshwater mollusc species shows that the same protective epitope is found in schistosome as well as in non-schistosome hosts. Moreover, it was also found on the haemocyanin of the keyhole limpet (*Megathura crenulata*), a carrier protein widely used in immunological studies.

Immunoprecipitation of antigens isolated from surface-radioiodinated miracidia using antibodies produced by rats during *S. mansoni* infection, reveals binding to surface components of M_r ranging from 30K to 200K (Fig. 1a). All these antigens contained the epitope defined by the protective monoclonal antibody (IPLSml)⁸ characterized previously on the 38K schistosomula surface molecule (Fig. 1b). These results show that the protective determinant is present in the different parasite stages on various molecules. Indeed, it has already been demonstrated that the IPLSml antibody recognizes high M_r ($\geq 150K$) components on the surface of cercariae⁷ and a 115K antigen in the metabolic products from adult worms¹².

Antigenic similarity between trematodes and their respective intermediate hosts has been described¹⁻⁵. We therefore studied the possibility of identity between characterized immunogenic *S. mansoni* antigens and *B. glabrata* components. Antibodies directed against soluble mollusc extract were raised in rabbits and used to immunoprecipitate surface-labelled antigens of miracidia and schistosomula. In both parasite stages, rabbit antibodies bound to surface molecules and the electrophoretic patterns of isolated antigens were similar to that obtained with the IPLSml antibody. The rabbit immunoglobulins bound strongly to the protective epitope of parasite target antigens as they were able to inhibit (up to 80%) the binding of IPLSml to the 38K schistosomula antigen in a solid-phase competitive assay⁹. It is important to note that antigenic identity between *B. glabrata* and *S. mansoni* was only associated with the 38K

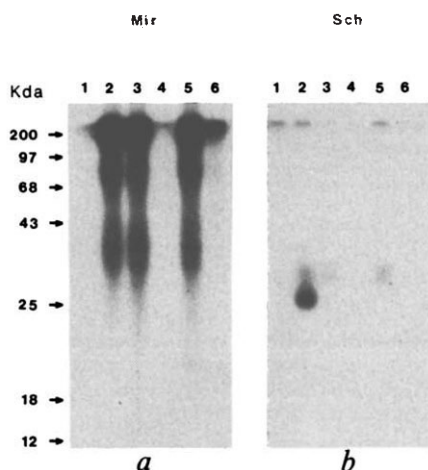


Fig. 1 Antigenic identity between *S. mansoni* target antigens and *B. glabrata*. A Puerto Rican strain of *S. mansoni* was used. Miracidia were prepared from eggs collected from livers of infected golden hamsters¹⁶. Schistosomula were obtained by passage of cercariae through isolated pieces of Swiss mouse skin¹⁷. Parasites were labelled by lactoperoxidase-catalysed radioiodination¹⁸ and antigens were isolated and analysed by SDS-PAGE as previously described¹⁹. *B. glabrata* (albino strain) bodies were ground in liquid nitrogen until a fine powder was obtained. The powder was dissolved in distilled water (8 ml g⁻¹) and sonicated at 4 °C for 4 × 15 s at 25 W. The soluble mollusc extract was obtained following a 1 h centrifugation at 100,000g at 4 °C. New Zealand rabbits were immunized by injection of 1 ml (5 mg protein) mollusc extract or 1 ml extract that was previously deglycosylated by the trifluoromethanesulphonic acid method of Edge *et al.*²⁰. Immunization was performed according to the technique of Vaitukaitis *et al.*²¹ in the presence of complete Freund's adjuvant. Surface antigens of miracidia (a) or schistosomula (b) were immunoprecipitated by normal Fischer rat serum (1), serum from Fischer rats infected by *S. mansoni* for 100 days (2), IPLSml monoclonal antibody (3), normal rabbit serum (4), anti-*B. glabrata* rabbit serum (5) or anti-deglycosylated *B. glabrata* rabbit serum (6).

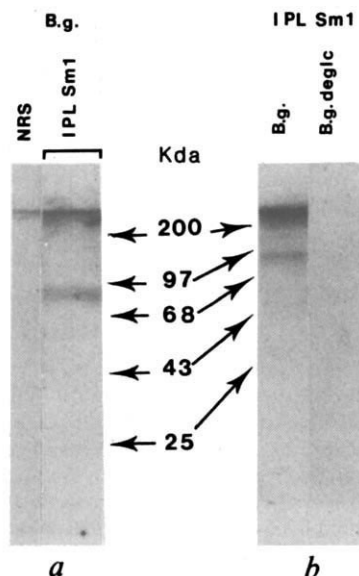


Fig. 2 Identification of cross-reactive *B. glabrata* components. a, *B. glabrata* extract (30 µg protein) was analysed by SDS-PAGE under reducing conditions²², then transferred on to nitrocellulose. Western blotting was performed according to the technique of Burnette²³ using 10 µl normal rat serum (NRS) or 10 µg IPLSml monoclonal antibody (two different preparations were used). Fixed antibodies were revealed after incubation with ¹²⁵I-labelled rabbit anti-rat IgG. b, SDS-PAGE analysis of deglycosylated²⁰ *B. glabrata* extract followed by Western blotting using IPLSml monoclonal antibody.

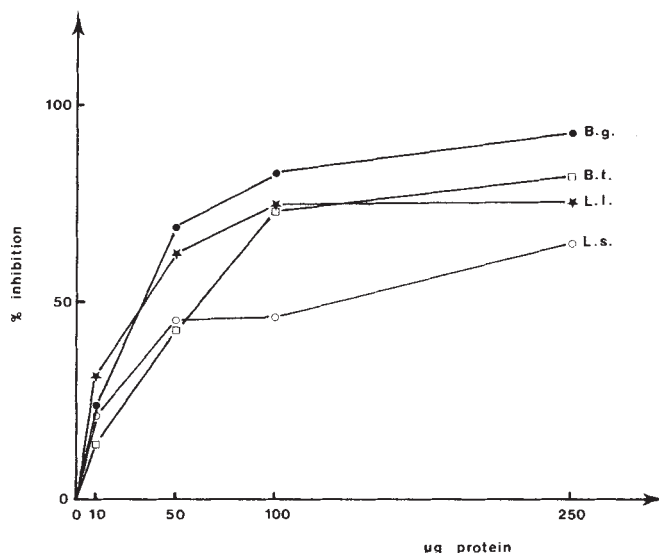


Fig. 3 Inhibition of IPLSml antibody binding to the 38K *S. mansoni* antigen by different fresh water mollusc extracts. Detergent extract from 250 ¹²⁵I-labelled schistosomula was incubated with 5 µg IPLSml antibody in the absence or in the presence of increasing concentrations (10, 50, 100 or 250 µg protein) of *B. glabrata* (B.g.), *Bulinus truncatus* (B.t.), *Lymnaea stagnalis* (L.s.) or *Lymnaea limosa* (L.l.) mollusc extracts. *B. truncatus* were obtained from the Pasteur Institute of Alger and *L. stagnalis* from the Paul Ehrlich Institute (Frankfurt, Germany). *L. limosa* were collected from local streams in the north of France. Immune complexes were analysed by SDS-PAGE¹⁹ and the amount of 38K antigen immunoprecipitated was determined by counting polyacrylamide gel slices containing the molecule. The percentages of inhibition were calculated as follows: $a - b/a$ where *a* and *b* represent, respectively, the amount of radioactivity isolated in the absence or in the presence of mollusc extract.

antigen among the surface schistosomula components recognized by infected rat antisera.

The observation that the IPLSml antibody bound to a number of molecular structures in the different developmental stages of the parasite suggested that the protective epitope could be found on carbohydrate chain(s) linked to different protein or proteolipidic structures. This was supported by work showing that rabbit antibodies produced against deglycosylated mollusc extract did not recognize surface-labelled parasite antigens. These antibodies were also unable to compete with the IPLSml antibody for binding to the 38K molecule (<5% inhibition was observed in solid-phase radioimmunoassay).

The identification of cross-reactive *B. glabrata* components was investigated by SDS-polyacrylamide electrophoresis (PAGE) of soluble mollusc extract followed by Western blotting using the IPLSml antibody. The antibody bound preferentially to a 90K *B. glabrata* component (Fig. 2). Several additional fainter bands with a variable intensity in different experiments are also seen, suggesting that the cross-reactive epitope could be found to a lesser extent on other molecular structures. The 90K band was no longer labelled when deglycosylated *B. glabrata* extract was analysed, confirming the glycanic nature of the epitope defined by IPLSml.

These data indicate that *S. mansoni* and its intermediate host share a common glycanic determinant previously demonstrated to be active in immunity to schistosomes^{8,9}. The presence of this epitope in metabolic products from adult worms has been described and the present results have shown that it is also distributed on various surface components of miracidia, that is, on the infective larvae before they have penetrated into the molluscan host. These two observations argue against the possibility that the 38K protective epitope on cercariae and newly-transformed schistosomula could result from the acquisition of

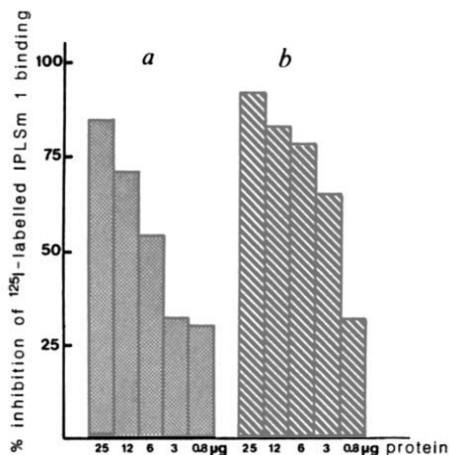


Fig. 4 Inhibition by KLH of radiolabelled IPLSm1 binding to *S. mansoni* antigen. Experiments were carried out on polyvinylchloride (PVC) microtitre plates coated with parasite antigen according to the method previously described⁹. Fifty microlitres of ¹²⁵I-labelled IPLSm1 antibody were simultaneously added to each well with in *a*, 50 μl various concentrations of purified keyhole limpet haemocyanin (KLH) (France Biochem, Meudon) [▨], or KLH deglycosylated by the method of Edge *et al.*²⁰ [■], and in *b*, 50 μl of native [▨], or deglycosylated [■], *B. glabrata* extract at different protein concentrations.

molluscan components by specific or non-specific adsorption and show that the synthesis of this carbohydrate moiety can occur both in the mollusc and in the parasite throughout its life cycle. Such results raise questions about the role of antigenic identity in host-parasite relationships and how it could arise. It has been supposed that the existence of cross-reactive antigens results from an adaptation of the parasite to its host and thereby prevents immune recognition. Indeed, in non-permissive mollusc hosts, immune killing of parasites by phagocytic cells has been described¹³ and is comparable to the antibody-dependent cellular cytotoxicity observed in vertebrate hosts¹⁴.

Previous studies using sera from patients infected by different trematodes indicate that the 38K schistosoma antigen could be specific for the *Schistosoma* genus¹⁰; we tested this by analysis of mollusc extracts from schistosome or non-schistosome host species. *B. glabrata* extracts strongly inhibited (up to 95% in the presence of 250 μg total protein) the immunoprecipitation of the labelled 38K antigen by IPLSm1 antibody (Fig. 3). *Bulinus truncatus* and *Lymnaea stagnalis*, which are the respective intermediate hosts for *Schistosoma haematobium* and *Trichobilharzia ocellata* (an avian schistosome), were also able to compete with the *S. mansoni* antigen, showing respectively, an 80 and 65% decrease of the isolated labelled 38K molecule for the same maximal dose of protein added. Moreover, a similar level of inhibition (75%) was observed with the non-schistosome host *Lymnaea limosa*. Extract from the land snail *Helix pomatia* did not compete with the 38K antigen (results not shown) and such data suggested that the protective epitope could be found in many freshwater snail species without restriction to schistosome hosts.

Further experiments showed that haemocyanin from the keyhole limpet (*Megathura crenulata*) also possesses the carbohydrate protective epitope. Figure 4 illustrates the competition of purified keyhole limpet haemocyanin for the binding of the IPLSm1 antibody to parasite antigens and demonstrates the role of carbohydrate moieties in the inhibition. Thus, these results show the expression in a human parasite of a major antigenic epitope whose origins are found in the marine mollusc *M. crenulata* (that has been in existence for several hundred million years) as well as in freshwater snails. The conservation of this oligosaccharide structure throughout evolution raises questions

of phylogeny and adaptation. It has been recently reported in various biological systems that membrane oligosaccharides are important in osmotic adaptation¹⁵ and it is tempting to speculate that important changes of osmolarity to which schistosomes are exposed during the rapid adaptation of free larval forms to invertebrate or vertebrate hosts might rely on this highly preserved structure. Work is presently in progress to explore the possible use of the oligosaccharide molecule in immunization against schistosomes.

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Germ-line transmission of genes introduced into cultured pluripotential cells by retroviral vector

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Embryonic stem cells isolated directly from mouse embryos¹ can be cultured for long periods *in vitro* and subsequently repopulate the germ line in chimaeric mice^{2,3}. During the culture period these embryonic cells are accessible for experimental genetic manipulation⁴⁻⁶. Here we report the use of retroviral vectors to introduce exogenous DNA sequences into a stem-cell line and show that these modified cells contribute extensively to the somatic and germ-cell lineages in chimaeric mice. Compared with current methods for manipulation of the mouse genome, this approach has the advantage that powerful somatic-cell genetic techniques can be used to modify and to select cells with germ-line potential, allowing the derivation of transgenic strains with pre-determined genetic changes. We have by this means inserted many proviral vector sequences that provide new chromosomal molecular markers for linkage studies in the mouse and that also may cause insertional mutations.

The XY stem cell line EK.CCE derived from the 129/Sv/Ev strain, was maintained *in vitro* as previously described¹. The cells were infected with the *mos*⁻¹neo retroviral vector⁷ produced



Fig. 1 *a*, V29.7 germ-line chimaera. Residual traces of the original albino host embryo component are limited to the small patch of white hairs on the head. *b*, 2A.5 germ-line chimaera.

free from replication competent helper virus by the Ψ 2 cell line⁸. Stem cells were infected by exposing them to a viral suspension. To ensure that a high proportion of the cells are infected and carry integrated vector this exposure was carried out repeatedly throughout an 8-day period. Sets of single-cell clonal lines were derived at various time points under non-selective conditions to determine the efficiency of infection. DNA from these clones was screened for vector sequences by Southern blotting. After 19 successive infections the clones are found to carry an average of 5 integrated vector sequences (range 1–15 copies). After 29 infections all the clones have a minimum of 10 copies (V29 series). A separate culture was exposed twice to a concentrated viral suspension (2A series).

To generate chimaeric animals, groups of 10–12 cells from the V29 and 2A polyclonal populations were reintroduced into blastocyst-stage embryos. From both cell populations the number of chimaeras obtained is high (20 out of 21 live-born animals). There is no difference between the infected EK.CCE cells and non-infected EK.CCE control cultures both in the efficiency of chimaera formation (Table 1) and in the extent of the contribution to the somatic tissues by the cultured stem cells (Fig. 1). The distortion in the sex ratio among the chimaeric population (85% male) is caused by conversion of XX host embryos into phenotypic males by the high efficiency of incorporation of the XY cultured cells^{2,3}. We test-bred 16 phenotypically male chimaeras and found that 13 animals are fertile, 6 of which are transmitting V29- and 2A-derived genomes to their F_1 progeny (Table 2).

To confirm that the introduced cells contain integrated proviral vectors, and that these are being transmitted to the F_1 generation, DNA samples from 12 individuals in the first litter sired by the 2A.5 chimaera and from 6 individuals sired by the V29.7 chimaera were analysed by Southern blotting. This analysis reveals many copies of vector DNA integrated at many different chromosomal locations in these F_1 mice (Fig. 2).

From the analysis of these individuals it is possible to obtain an estimate of the minimum number of different proviral vector

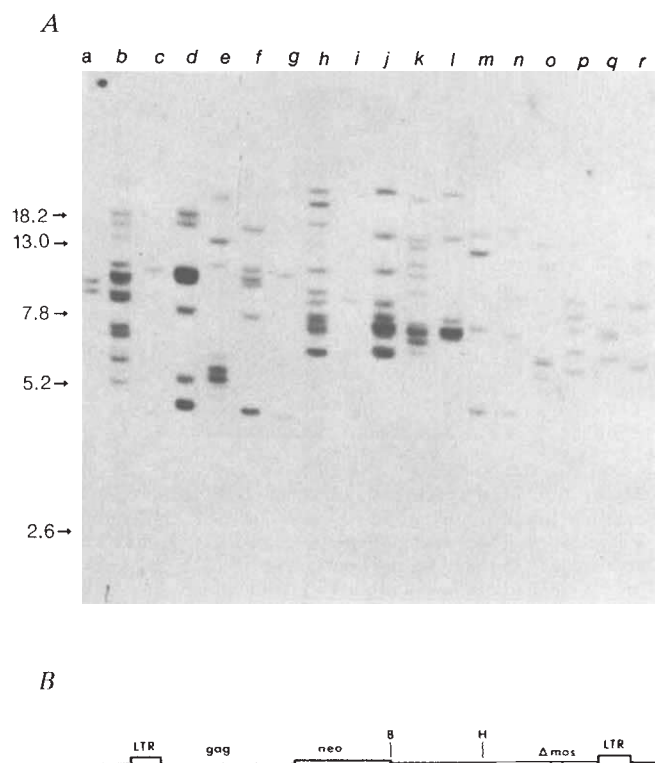


Fig. 2 *A*, Southern blot analysis of DNA samples from F_1 offspring of two germ-line chimaeric males. Samples are distributed as follows. *A*, V29.7 progeny; lanes *a*, *g*, V29.7.1; *b*, *h*, V29.7.2; *c*, *i*, V29.7.3; *d*, *j*, V29.7.4; *e*, *k*, V29.7.5; *f*, *l*, V29.7.6. 2A.5 progeny; lanes *m*, *p*, 2A.5.10; *n*, *q*, 2A.5.11; *o*, *r*, 2A.5.12. *B*, *Bam*HI; *H*, *Hind*III restriction sites; LTR, long terminal repeat. Single line, chromosomal DNA; double line, vector DNA.

Methods. *A*, The two restriction enzymes used here cleave once in the proviral vector outside the region of homology with the pSV2neo probe, consequently each restriction fragment on the autoradiograph corresponds to a fragment containing a unique integration site of a proviral vector genome. DNAs were prepared from whole newborn animals (V29.7 progeny) or from livers from 1-week-old animals (2A.5 progeny). Samples (3 μ g) were digested with *Bam*HI (lanes *b–g*; *m–o*) or with *Hind*III (lanes *a*, *h–l*, *p–r*), fractionated on 0.7% agarose gels, transferred to nylon membranes, hybridized with nick-translated pSV2neo, washed and autoradiographed using standard procedures.

loci that are transmitted to the F_1 generation. There are at least 24 unique integration sites represented in the 12 F_1 animals from the 2A.5 male. Estimation of the numbers of integrated vector sequences in the F_1 progeny of the V29.7 chimaera is more difficult because of the larger number of vector inserts, but at least 39 unique insertion events are represented in the 6 F_1 progeny analysed from the V29.7 chimaera. The pattern of the transmission of the vector loci, taking into account the randomizing influence of meiosis, suggests that at least two distinct tissue culture cells have contributed to the germ-line transmission in both individuals.

Our results demonstrate that cultured embryonic stem cells provide an efficient means for the production of transgenic animals. This method is an important addition to techniques of introduction of foreign DNA into the mouse germ line by direct injection into pronuclei of fertilized eggs⁹ or by retroviral infection of embryos¹⁰.

The use of pluripotent stem cells to create transgenic mice offers the advantage that a range of techniques to manipulate the tissue-culture genome, including gene transfer or mutagenesis and selection, can be applied. Cell clones can be

Table 1 Construction of chimaeras from non-infected and retrovirally infected stem-cell lines

Cell line	No. of embryos injected	No. of animals born	No. of chimaeric animals	Chimaeras		Set-up	Males	
				Male	Female		Bred	Germ-line
CCE non-infected	33	17 (52%)	17 (100%)	15	2	15	13	5
CCE.V29	31	14 (45%)	13 (93%)	12	1	11	8	5
CCE.2A	18	7 (38%)	7 (100%)	5	2	5	5	1

The EK.CCE cell line was derived from a single XY blastocyst-stage embryo of the 129/Sv//Ev strain (homozygous for the black agouti coat colour markers and the GPI-1^b allele) and maintained on feeder layers of STO cells¹. Before the infection experiment, to monitor possible effects resulting from the infection protocol, stem cells from the culture were tested for their ability to form chimaeras by reintroducing 10–12 individual cells into host MFI strain blastocysts (albino; homozygous for the GPI-1^b allele)². All of the resulting offspring were scored as chimaeric by the presence of coat-hair and eye pigment (contribution from the donor cells approached 100%). Fifteen phenotypic male animals were test-bred with albino MFI females. Of the 13 fertile animals 5 (38%) show transmission of the cultured cell genome to the F₁ progeny, demonstrating that the CCE line is capable of efficient colonization of the germ line. The cell line was thawed and the cell population infected using medium from cultures of the MPSV mos⁻¹neo Ψ2 (ref. 7) producer cell line. The CCE.V29 cell culture was obtained by a serial infection procedure using freshly collected culture supernatant collected from confluent dishes of Ψ2 producer cells (12-h collection period). Supernatant, filtered through a 0.45-μm Millipore filter and supplemented with 10% fetal calf serum and 1 μg ml⁻¹ polybrene, was added to the stem cell culture at 6–8 h intervals over an 8-day period, during which time the culture was passaged once onto fresh feeder plates. Single cell clonal lines were derived from the culture after 19 and 29 rounds of infection (V19 and V29, respectively) and the clones analysed by Southern blotting of genomic DNA using pSV2neo as the probe. The CCE.2A culture was obtained by treating a culture of CCE stem cells with a 50-fold concentrated viral supernatant²⁰. The culture was infected twice at an interval of 6 h. The V29 and 2A cultures were grown for 3 days and 24 h, respectively, under non-selective conditions, before reintroduction of stem cells into host blastocysts. From both series chimaeric animals were generated at a high efficiency (20 out of 21 animals) and many of these are phenotypically male (17 male, 3 female), a result similar to that seen for the uninfected CCE control series. The male chimaeras were set up for test breeding. Results show that the infection procedure has no effect on the differentiation ability of the cultured stem cells: 5 out of 8 fertile males in the V29 series and 1 out of 5 fertile males in the 2A series are germ-line chimaeras.

Table 2 Breeding data from germ-line chimaeras from virally infected stem cells

Animal	No. of litters	No. of progeny black agouti	No. of progeny (albino)	Female	Male
CCE.V29.2	3	27	0	14	13
CCE.V29.4	4	4	40	19	25
CCE.V29.5	2	17	0	9	8
CCE.V29.7	3	41	0	20	21
CCE.V29.9	2	22	0	9	13
CCE.2A.5	2	25	0	12	13

Phenotypically male chimaeras were caged with virgin MFI females homozygous for the GPI-1^b allele and the albino locus. The resulting litters were scored for progeny carrying the dominant black agouti phenotype carried by the CCE cell line. GPI analysis of blood samples taken from black agouti progeny confirmed the transmission of CCE.2A and CCE.V29 derived GPI-1^b allele. Six males show transmission of the culture-derived component. Five of the animals exclusively transmit sperm derived from the CCE cell lines. This shows that, although overtly male, 5 of the 6 germ-line animals are fertile sexual mosaics in which the albino-host-derived XX cells are unable to contribute to the functional sperm population^{2,3}. Twelve offspring (3 female, 9 male) from the 2A.5 animal and 6 from the V29.7 animal (3 female, 3 male) were killed at, or shortly after, birth and genomic DNA samples prepared for Southern blotting analysis.

selected for specific phenotypes and fully characterized before being used to make transgenic chimaeras.

We have used a retroviral vector to mediate gene transfer into these stem cells. The unique advantages of retroviral vectors have been demonstrated in several systems (refs 11–14; see ref. 15 for review). In particular they provide a very efficient means of introducing single copies of novel gene constructs into many chromosomal sites. By controlled exposure of a stem-cell culture to helper-free virus we have been able to generate various populations of cells that have an average copy number of between 1 and 20 random vector integrants. The high efficiency of this gene transfer technique obviates the need to select for introduced DNA.

We used the high copy number-containing cells to generate six transgenic chimaeras. From only two of these germ-line animals we have characterized 63 novel viral integration loci from the transgenic F₁ offspring of the first litters. We estimate from this experiment alone that we will have generated >200 novel integration sites in the mouse genome, which compares favourably with the frequency of acquisition of new germ-line proviruses observed to occur spontaneously in specific intercrosses of mouse strains caused by mobilization of endogenous viral genomes¹⁶. Both these systems are efficient at producing high numbers of integration sites and it is likely that novel insertional mutations will be generated. To facilitate this, our approach is to use stem cells predetermined as carrying many vector copies. Breeding studies will enable identification and

mapping of X-linked, dominant and recessive mutations. Mutations caused by insertion of a characterized DNA sequence can also be studied at the molecular level following cloning of the integration site¹⁷. Another advantage of the use of proviral vectors carrying bacterial selection markers is that they allow rapid cloning^{18,19} of any insertionally mutated gene revealed by these breeding studies.

In addition the introduction of many randomly distributed proviral vector sequences provides a set of chromosomal markers for linkage studies in the laboratory mouse. These markers will offer a link between classical and molecular genetic analysis.

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Note added in proof: We now have a total of 13 germ-line chimaeras constructed using MPSV mos⁻¹neo infected EK.CCE cells, including two males made from clonal lines which were selected in culture for resistance to G418.

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A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria

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Many biological processes are coupled to ATP hydrolysis. We describe here a class of closely related ATP-binding proteins, from several bacterial species, which are associated with a variety of cellular functions including membrane transport, cell division, nodulation in *Rhizobium* and haemolysin export. These proteins comprise a family of structurally and functionally related subunits which share a common evolutionary origin, bind ATP and probably serve to couple ATP hydrolysis to each of these biological processes. This finding suggests a specific role for ATP in cell division, nodulation during nitrogen fixation and protein export, and allows us to assign a probable function to one of the protein components from each of these systems.

Figure 1 shows an alignment of 10 sequences to illustrate the extensive homology between the various members of this family of bacterial proteins. The sequences are from nine independent proteins, as the RbsA protein has two distinct but homologous domains. Six of these proteins are transport components, the others being associated with cell division (FtsE), nodulation in *Rhizobium* (NodI) and haemolysin export (HlyB). The homologies between these nine proteins are even more striking considering that the sequences are derived from three different species. The physical relationships between the genes encoding these homologous proteins and adjacent genes of related function are shown diagrammatically in Fig. 2.

The considerable similarity between these nine proteins implies that they serve related functions within the cell. What might this function be? There is now good evidence that at least some members of this family of proteins bind ATP. Six of the proteins, OppD, OppF, HisP, MalK, PstB and RbsA, are components of periplasmic binding-protein-dependent transport systems and these proteins are among those most extensively

Table 1 Alignment of various prokaryotic and eukaryotic sequences to illustrate the conservation of amino acids in the nucleotide-binding sites of these proteins

Protein	Species	Sequence
OppD	<i>S. typhimurium</i>	GETLGVESGGKSSLR
OppF	<i>S. typhimurium</i>	GESGVVSGGKSFAR
HisP	<i>S. typhimurium</i>	GDVISIISGGKSFLLR
MalK	<i>E. coli</i>	GEFVVFVPSGGKSTLLR
PstB	<i>E. coli</i>	NQVTFIIPSGGKSTLLR
RbsA(N)	<i>E. coli</i>	GRVMAVLENGAGKSTMMK
HlyB	<i>E. coli</i>	GEVIGIVSGGKSTLTK
NodI	<i>R. leguminosarum</i>	GECPVLENGAGKSTTR
FtsE	<i>E. coli</i>	GEMAFITISGAGKSTLLK
ATPase	<i>E. coli</i>	GORELIIDRDTKTAIAI
ATPase	<i>E. coli</i>	GCKVGLFPGACVGVNMM
RecA	<i>E. coli</i>	GRIVEIVPESSGKSTLLR
UvrD	<i>E. coli</i>	RENLLVLNAGSGKSTRLV
ATPase	Bovine	GCKIGLFLPACVGVNGLM
Adenylate Kinase	Rabbit	SKTIFPVVPGSGKSTLQCE
Myosin	Rabbit	NQSILITSSGAGKSTNTK
EF-Tu	<i>E. coli</i>	HNNVGTIHDVIGKTLTA
EF-G	<i>E. coli</i>	YRNIGISLIDAGKSTERI
v-ras (Harvey)		EYKLVVVEARGVKSALTI
v-ras (Kirsten)		EYKLVVVEASGVKSALTI
pBJ Bladder carcinoma transforming		EYKLVVVEARGVKSALTI
pBJ Bladder carcinoma cellular		EYKLVVVEAGVKSALTI

The consensus sequence⁷ is boxed. See refs 12 and 31 for references to these sequences and for more extensive listings.

characterized¹⁻³. Preliminary sequence data indicate that components of the glycerol 3-phosphate and arabinose transport systems also belong to this family of proteins (J. Tomassen and R. Hogg, personal communications). It therefore seems likely that all binding-protein-dependent transport systems will require such a component. It has been shown that binding-protein-dependent transport systems are distinct from other transport systems in that they are probably energized by the direct hydrolysis of ATP or a closely related nucleotide rather than by the electrochemical proton gradient^{4,5}. Nevertheless, direct evidence of a role for ATP has been obtained only recently. The OppD, HisP and MalK proteins, from the oligopeptide, histidine and maltose transport systems, respectively, have now unambiguously been shown to bind ATP; the OppD protein can be specifically labelled with the ATP-affinity analogue 5'-*p*-fluorosulphonylbenzoyl adenosine¹, the HisP and MalK proteins react with the ATP-affinity analogue 8-azido-ATP⁶ and the purified MalK protein will bind ³²P-ATP (H. Nikaido, personal communication). It therefore seems probable that these proteins serve to couple ATP hydrolysis to transport. However, as it has not yet been possible to demonstrate ATP hydrolysis by any of these proteins, the possibility that ATP-binding serves a purely regulatory role cannot be eliminated.

Besides the transport components, the other proteins identified here all possess a potential nucleotide-binding site. A short consensus sequence, present in a wide variety of nucleotide-binding proteins, was first discussed by Walker *et al.*⁷. Crystallographic analysis of adenylate kinase and several other enzymes has shown that this conserved sequence forms the phosphate-binding region^{8,9}. Many nucleotide-binding proteins from both prokaryotes and eukaryotes retain this sequence, including kinases, ATP hydrolases and the GTP-binding *ras* gene product p21 (Table 1). Each of the proteins described in the present study retains such a consensus sequence (Table 1) which is characteristic of a mononucleotide-binding site¹² and is located in one of the regions most highly conserved between the various proteins. It is important to note that while most bacterial proteins that bind nucleotides, such as elongation and initiation factors, RecA¹⁰ or UvrD¹¹, also retain this short consensus sequence, such proteins share no additional homology. The proteins described here are clearly distinct from all other nucleotide-binding proteins in that they show extensive conservation of amino-acid sequence which extends over almost the entire length of each protein. It seems highly likely that each of these proteins will bind and hydrolyse ATP or a related nucleotide.

What are the biological implications of the homologies between the ATP-binding subunits of periplasmic transport systems and the HlyB, FtsE and NodI proteins? Binding-protein-

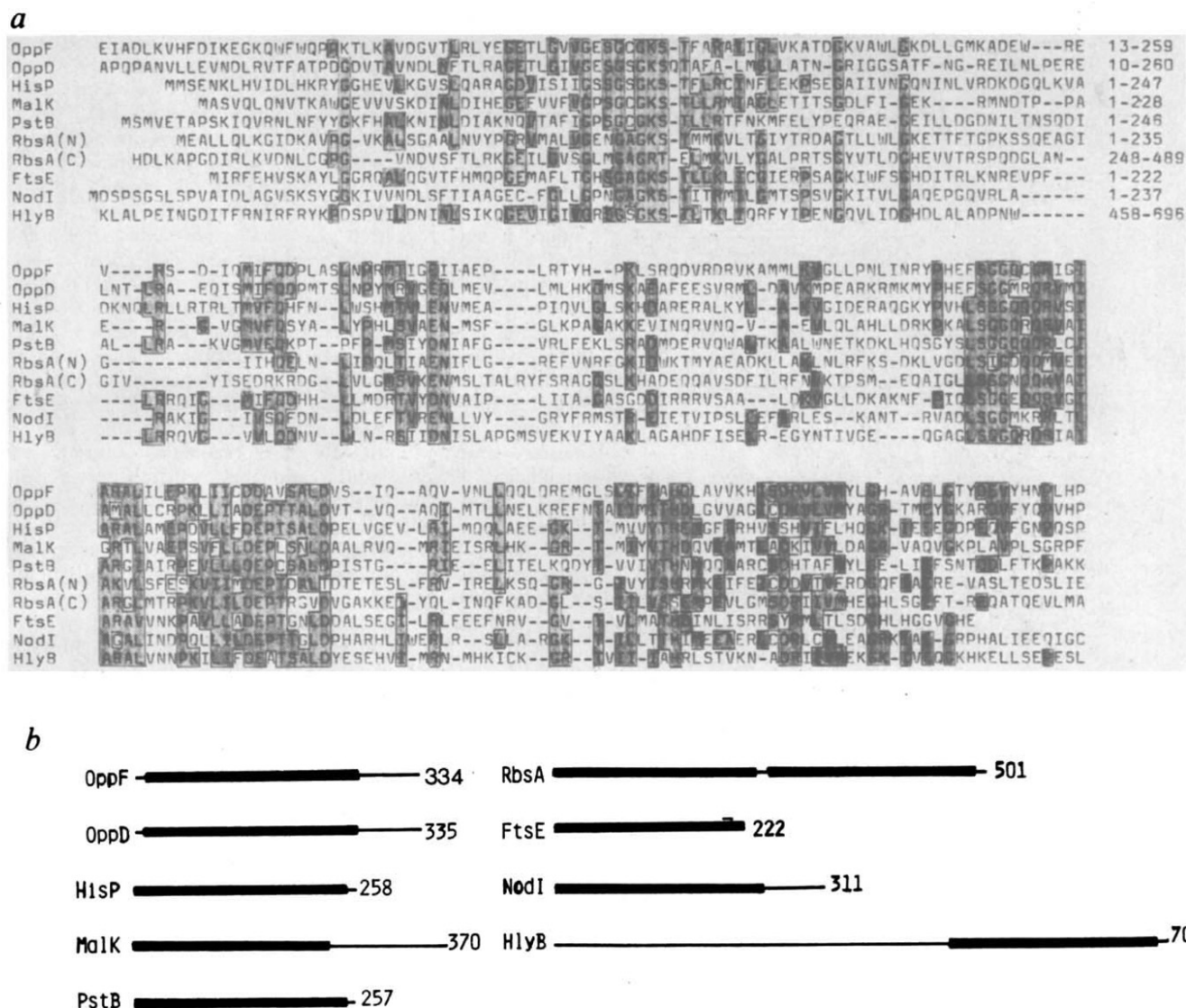


Fig. 1 Sequence homologies between bacterial ATP-binding proteins. **a**, Proteins are aligned to illustrate their homology. Conserved amino acids are boxed and shaded. Sequences are from the following sources: OppD and OppF, *Salmonella typhimurium* oligopeptide permease^{1,13}; PstB, *E. coli* phosphate transport²³; HisP, *S. typhimurium* histidine transport¹⁴; MalK, *E. coli* maltose transport²⁶; RbsA, *E. coli* ribose transport²⁹; NodI, *Rhizobium leguminosarum* nodulation³⁰; HlyB, *E. coli* haemolysin secretion¹⁵; FtsE, *E. coli* cell division³¹. **b**, Location of the conserved domain whose sequence is presented in **a** (thick line) is shown in relation to the rest of the protein (thin line). The number at the end of each line indicates the number of amino-acid residues in that protein.

dependent transport systems are multicomponent and all have a similar organization, involving a periplasmic substrate-binding protein and two highly hydrophobic integral membrane components in addition to the conserved ATP-binding subunit^{2,3,13,14}. There is no evidence that the HlyB, FtsE or NodI proteins are related to periplasmic transport processes. It is, of course, possible that the phenotypes of *ftsE* and *nodI* mutations could be secondary consequences of defects in specific transport systems. However, the other proteins encoded by the operons which encode FtsE and NodI show little or no resemblance to components of binding-protein-dependent transport systems. Thus, none of the genes linked to *nodI* (*nodABC*, *nodJ*) or to *ftsE* (*ftsXY*) (Fig. 2) encode a protein with a signal peptide and cannot therefore encode a periplasmic protein. In addition, NodA, NodB and FtsX are highly hydrophilic and although NodJ, NodC and FtsY are hydrophobic they possess none of the characteristic features of the integral membrane components of periplasmic transport systems¹³. Thus, if NodI and FtsE are components of specific transport systems, these must be very

different in organization and function from the typical binding-protein-dependent systems. This view is supported by the functions of the HlyB protein. It is well established that only four closely linked genes are required for haemolysin secretion¹⁵⁻¹⁸. The *hlyA* gene encodes haemolysin itself while *hlyC* encodes an enzyme required for activation of haemolysin before its secretion from the cell. The other two proteins, HlyB and HlyD, are required for export of haemolysin from the cell. Such a well-defined role entirely precludes the HlyB protein from being a component of a periplasmic transport system. This therefore suggests that the role of these nucleotide-binding proteins may be to couple energy, via ATP hydrolysis, to a variety of distinct biological processes including, but not restricted to, transport processes.

The relative molecular masses (M_r s) of these ATP-binding proteins vary from ~25,000 (FtsE, PstB, HisP) to 66,000-79,000 (HlyB)^{15,19}. The basic domain which is conserved in all the proteins is of M_r ~25,000 and the HisP, FtsE and PstB proteins consist solely of this domain (Fig. 1b). The OppD, OppF, NodI

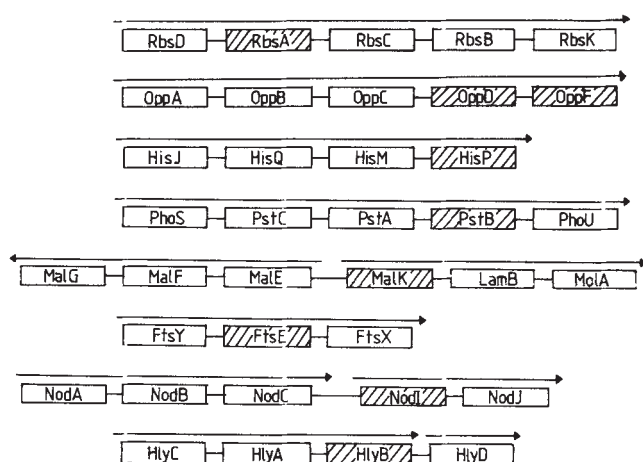


Fig. 2 Scheme showing the location of ATP-binding proteins with respect to adjacent genes (not to scale). The arrows indicate the direction and extent of transcription. Genes encoding the ATP-binding proteins are hatched. Details of the function of the other genes is discussed in the text and can be obtained from the following sources. Opp, oligopeptide permease¹³; Rbs, ribose transport²⁹; His, histidine transport¹⁴; Mal, maltose transport²⁸; Pst, phosphate transport²³; Hly, haemolysin synthesis and secretion^{15,16,32}; Nod, nodulation in *Rhizobium*^{30,33}; Fts, cell division³¹.

and MalK proteins have, in addition to this conserved domain, a variable number of amino acids at their C-terminus. These C-terminal extensions are generally rather short and, because HisP, PstB and FtsE contain no such extensions, it seems unlikely that these sequences are involved in conferring specificity on the various proteins. However, it is interesting to speculate that the rather longer C-terminal extension of the MalK protein (120 amino acids) may be important for the regulation of maltose transport, as the MalK protein has been implicated in interactions with factor III^{Glc} during inducer exclusion (M. H. Saier, personal communication). Only the HlyB protein possesses what is clearly an additional functional domain. This is an N-terminal extension of $M_r \sim 50,000$; its role in the specific function of the HlyB protein is unknown.

What is the subcellular location of these nucleotide-binding proteins? The relatively hydrophilic HisP²⁰, MalK^{21,22}, PstB²³ and OppD¹ proteins are all peripherally associated with the cytoplasmic membrane, possibly via an interaction with a second transport component²². The HlyB protein is also thought to be membrane associated^{19,24}, although reports that it is in the outer membrane²⁴ now seem unlikely (N. Mackman and I.B.H., unpublished results). Preliminary evidence suggests that FtsE is also membrane associated (D.R.G. and G.P.C.S., unpublished results), while nothing is known about the subcellular location of NodI. Thus, although these ATP-binding subunits are often associated with membrane-related functions, we cannot determine whether or not they are also involved in cytoplasmic processes. It is possible that each of the ATP-binding proteins functions as a dimer or larger oligomer, and that the sequences that are conserved between the proteins, other than the ATP-binding site itself, are involved in such multimerization. For the Opp oligopeptide transport system, two ATP-binding subunits, OppD and OppF, are required for transport¹³. In the ribose transport system, the equivalent proteins have apparently fused into a single, two-domain protein, each domain of which possesses a potential ATP-binding site. Because of the many similarities between the binding-protein-dependent systems it

seems reasonable to suppose that the other transport systems will also require two ATP-binding domains. Thus, the HisP and MalK proteins may well function as homodimers or possibly as larger oligomers. There is also some indication that the *hlyB* gene product may exist as a dimer since the *hlyB* gene is translated from two separate start points to give distinct products with M_r s 46,000 and 66,000¹⁶; these two polypeptides share the same C-terminal domain, including the ATP-binding site.

When the homology between HisP and MalK was first identified, it was suggested that this might reflect a common evolutionary origin for the binding-protein-dependent transport systems^{3,25}. However, now that the sequences of all the components from each of four different transport systems have been obtained^{2,13}, this explanation for the homology seems less likely. The binding proteins and hydrophobic integral membrane components from the different transport systems differ considerably in size and show no extensive primary sequence homology (although the binding proteins do assume a similar tertiary structure^{26,27}). Only the ATP-binding subunits show significant sequence conservation between transport systems. The extensive nature of this homology, together with the present finding that equally closely related ATP-binding subunits are associated with functions apparently unrelated to periplasmic transport systems, implies that these subunits had a common evolutionary origin which may well be distinct from that of the other transport components.

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Note added in proof: Since submitting this paper a protein of unknown function encoded by the *MbpX* gene of liverwort chloroplast DNA (Ohya, K. *et al. Nature* **322**, 572-574; 1986) has been found to show extensive homology to the proteins described here. The accompanying paper by Doolittle *et al.* describes an additional closely-related protein, UvrA from *E. coli*.

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Domainal evolution of a prokaryotic DNA repair protein and its relationship to active-transport proteins

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The ABC excision nuclease of *Escherichia coli* is an ATP-dependent DNA repair enzyme composed of three protein subunits, UvrA, UvrB and UvrC. The DNA sequences of all three genes have been reported¹⁻³. UvrA, the component that binds directly to the DNA, and UvrB, which attaches itself to the UvrA-DNA complex, both contain consensus sequences thought to be diagnostic of ATP-binding sites⁴, although the UvrC sequence does not. We now report that a computer analysis of the UvrA sequence has revealed an unusual series of internal duplications centering around putative metal-binding sites which may be involved in the interaction with DNA. We also find a strong evolutionary relationship to a family of prokaryotic membrane-associated active-transport proteins.

The UvrA sequence is composed of 940 amino acids that can be divided roughly into three homologous units, each containing sequences that are recognizably similar to the A and B consensus sequences postulated by Walker *et al.*⁴ to be characteristic of ATP binding. What distinguishes the UvrA sequence further is that each of the segments is set off from the others by a third set of units, two of which have sequences recently found to be associated with DNA binding and metal binding^{5,6}. In particular, the sequence CXXC-(X)_n-CXXC occurs twice in UvrA. An isolated 'half-unit', CXXC, also occurs, the apparent vestige of a partial duplication.

The three homologues of the UvrA sequence also strongly resemble a family of membrane-associated transport proteins that have themselves been found to bind ATP and also contain the A and B consensus sequences⁷⁻¹⁰. Unlike most other occurrences of the alleged ATP-binding sequences, the similarities between the two types of protein extend beyond the short sequences denoted A and B, and a case for common ancestry can be made with statistically expressible confidence. Indeed,

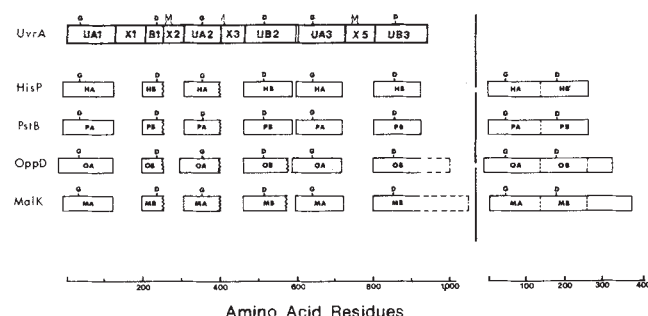


Fig. 1 Block diagram showing major features of 940-residue UvrA protein and regions where prokaryotic active-transport protein sequences can be aligned. Glycine residues in the characteristic 'GKS' sequence of 'type A' ATP-binding segments are denoted by 'G'. The 'essential' aspartic acid residue found in 'type B' segments is denoted by 'D'. Putative metal-binding sites are indicated by 'M'. Segments in UvrA that are not found in prokaryotic proteins are labelled as X1, X2, etc. The full sequence blocks of five prokaryotic transport proteins, which are composed of both 'type A' and 'type B' halves, are shown on the right side at the same scale.



Fig. 2 Optimal alignment of two portions of UvrA sequence with membrane-associated transport proteins HisP (ref. 8) and PstB (ref. 10). The latter two proteins lack the portion of the sequence thought to be characteristic of metal binding (CXXC-(X)_n-CXXC). Asterisks denote positions at which the two types of protein (UvrA and prokaryotic transport) have identical residues in at least one of the cross-comparisons. The two arrows mark the ends of segments A1 and A3 and the beginnings of B2 and B3, respectively. The 280-residue section shown with a dashed line contains remnants of 'type A' and 'type B' segments (A2 and B1), as well as the putative zinc-binding segment encompassing 'finger 1'.

each of the transport proteins can be divided into two portions that can be aligned with various segments of the UvrA sequence, but not with the interrupting segments containing the putative zinc-binding 'fingers' (Fig. 1).

As an example, the histidine transport protein HisP from *Salmonella*, which is 258 residues long, can be divided into two portions, residues 1-131 containing the 'GKS' ('A site') and residues 132-258 containing the invariant aspartic acid thought to signal the 'B site'. These sequences are 25% and 32% identical with UvrA residues 609-727 and 808-940, respectively (Fig. 2). When these alignments were subjected to statistical jumbling¹¹, the authentic alignment scores were, convincingly, +3.7 and +9.8 standard deviations above the mean scores of the randomized alignments. In this part of the UvrA sequence the intruding metal-binding segment ('finger 2') is situated directly between the two segments that correspond to the two halves of the prokaryotic transport proteins (Fig. 2). In the case of the other potential zinc-binding site ('finger 1'), the segment occurs at the junction of a 'type B' and a 'type A' segment (Fig. 1). The other membrane-associated transport proteins whose sequences have been reported include the maltose K protein (MalK)⁸, the oligopeptide D protein (OppD)⁹ and the phosphate B protein (PstB)¹⁰, all of which have sequences that are not only homologous to each other, but also clearly resemble the duplicated UvrA segments.

The consensus sequences suggested by Walker *et al.*⁴ are exceedingly liberal, and the conditions are probably met by many proteins that may not bind nucleotides¹². Given the looseness of the consensus and the low level of resemblance among many families of nucleotide-binding proteins, it has usually been impossible to distinguish between divergence from a common sequence and functional convergence by judicious amino-acid replacement. Thus, although there has never been any doubt that the four prokaryotic transport proteins cited all originated from a common ancestor⁸⁻¹⁰, previously it has not been possible to extend the relationship to other families of ATP-binding

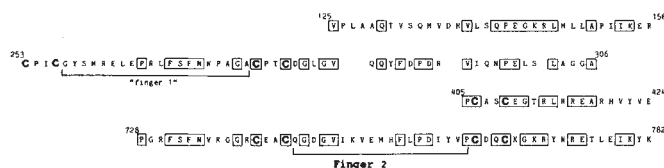


Fig. 3 Four segments in UvrA not found in prokaryotic transport proteins and which separate 'type A' and 'type B' segments. Identical residues are boxed.

proteins, putative or proven. It has been suggested that the MalK sequence is related to NAD dehydrogenase¹³, but we have re-examined this case with our computer programs and found it to be, at best, of marginal significance.

The question naturally arises of whether the transport proteins lost the 'finger-like' sequence or whether the UvrA protein acquired it. Further, if the latter is the case, was the sequence established gradually by unitary amino-acid replacements, or was it acquired as a functionally active unit from some other source? The various intruding segments themselves seem to be homologous (Fig. 3), although evidently portions have been lost during partial duplications. The implication is that the intruding sequence was originally acquired as a full-sized segment. Moreover, the proximity of the breakage and reunion points to the various CXXC sites suggests that this constellation of amino acids may have been especially prone to recombination events, either through general mispairing of homologous base sequences or as a result of its being a specific recombination site.

In theory it should be possible to reconstruct the history of events that led to the two systems (prokaryotic transport and DNA repair) on the basis of the similarity between the various alignable sequences. Figure 4 gives a scenario that shows how these sequences could have become situated at both the A/B and B/A junctions. It seems clear that the most recent major event in UvrA evolution was an internal duplication that led to residues 405-597, on the one hand, and 762-940, on the other, which include 'type B' units and most but not all of the intruding segment (Figs 1, 4). These two segments are now 40% identical. If we trace events further backwards, the next most likely duplication would have led to the segments denoted A1 and A3, segments that today are still 35% identical. Given that the corresponding active transport protein segments are 20-35% identical with the corresponding UvrA segments, it would seem reasonable to estimate that they last shared common ancestry at an even earlier stage. In this regard, the prokaryotic transport proteins themselves are 25-35% identical.

We therefore conclude that a gene for one of the prokaryotic transport proteins was duplicated, after which one of the duplicons was interrupted by a putative metal-binding gene (Fig. 4). This being the case, it might be possible to identify another prokaryotic protein that, through one genetic event or other, was the source of the intruding segment. A search of available sequences has not revealed any very obvious candidates, although, as was recently reported⁶, several other prokaryotic proteins have sequences of the CXXC-(X)_n-CXXC sort, including DNA primases from bacteriophage T7 (ref. 14) and *E. coli*¹⁵ and several (but not all) transfer RNA-aminoacyl synthetases⁶.

The structural and functional implications of these segmental intrusions should not be underestimated. For one thing, the segments containing the type A and B sites, whether in prokaryotic transport proteins or in UvrA, must fold independently and, as such, may legitimately be referred to as domains. Beyond that, the incorporation of a finger offers the opportunity for DNA binding, a major functional modification. The ATP-binding proteins of the prokaryotic active transport system do not seem to interact with DNA; rather, they are thought to bind to the cytoplasmic faces of the appropriate membrane-spanning

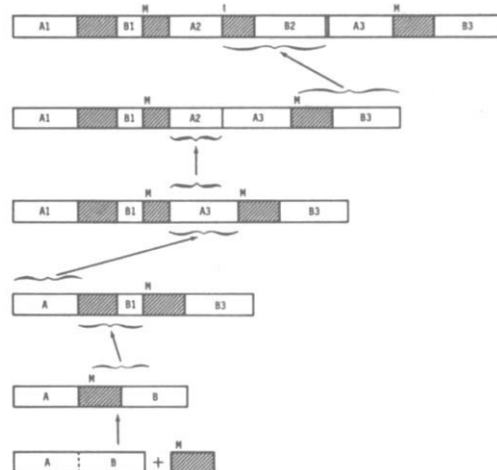


Fig. 4 Scenario consistent with present-day UvrA sequence. Existence begins with a typical prokaryotic transport protein (~260 residues) containing 'type A' and 'type B' segments. Acquisition of a metal-binding segment (~80 residues, shaded sections) intruding between the A and B units leads to a series of partial duplications. The progression shown here is reconstructed on the basis of units B2 and B3 having the most similar sequences, A1 and A3 the next most, and so on. Other schemes, less parsimonious, may more accurately reflect events.

proteins, changing the conformations of cooperative channel proteins depending on whether ATP or ADP is bound, and thereby providing the actual driving energy for the transport process¹⁶. In contrast, the UvrA protein binds to both single-stranded and double-stranded DNA and, when complexed with UvrB and UvrC, cuts damaged DNA at two sites separated by 12 or 13 nucleotides¹⁷. The two alleged metal-binding sites are far enough from one another for us to postulate that each of the two 'fingers' is in contact with one of the two incision sites. It will be of interest to determine if the UvrA subunit is responsible for the nucleophilic attack on the two phosphodiester bonds, which are offset by about 45 Å, and whether the ATP-binding property of the original protein has been recruited to aid in the process.

In support of the present findings, at least two atoms of zinc per mol of Uvr protein have now been found. Also, we have discovered a connection between the ABC excision nuclease and eukaryotic excision-repair enzymes in that a search of the recently published¹⁸ sequence of human ERCC-1 has revealed that the carboxy-terminal 60 residues of that protein are clearly homologous with the corresponding carboxy-terminal segment of UvrC.

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Mapping of the class II region of the human major histocompatibility complex by pulsed-field gel electrophoresis

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The class II region of the human major histocompatibility complex (MHC) encodes a polymorphic set of cell surface glycoproteins involved in the regulation of the immune response¹. Each glycoprotein is a heterodimer composed of a α -chain of relative molecular mass (M_r) 34,000 (34 K) and a β -chain of M_r = 28K. The products of the class II region have been characterized by the mixed lymphocyte reaction², serology³, primed lymphocyte typing⁴ and DNA cloning⁵⁻⁹. DR, DQ and DP, three subregions containing both α - and β -chains, and two additional loci, DZ α and DO β , locate this gene cluster on the short arm of chromosome 6. The precise genomic organization of these loci have been difficult to determine. Here we describe the use of pulsed-field gel electrophoresis¹⁰ together with restriction endonucleases having few genomic restriction sites and Southern blotting, to determine the order of the subregions and to derive a map for the human class II region. The order of these loci is similar to that of the homologous loci in the murine class II region¹¹. Our study establishes the use of pulsed-field gel electrophoresis in mapping large regions of the genome in higher eukaryotes.

The relative positions of only two subregions have been previously determined with certainty. Recombinants in family studies indicate that the DP subregion is centromeric to the DR subregion⁴. Studies of deletion mutants confirm the location of DP¹². Recombination events between the DQ and DR subregions have not yet been identified. Although the localization of both DO and DZ remains uncertain, these genes map in the HLA-D region¹³. Cosmid cloning studies provide maps of individual subregions¹⁴⁻¹⁸ but fail to link them, possibly because of the large distances between these loci, or because of repeated sequences¹⁸ which make cosmid 'walking' difficult. The technique of pulsed-field gel electrophoresis provides an opportunity to study the genomic organization of this region in a new way. It has permitted us to separate fragments of DNA up to 1,000 kilobases (kb) in size on agarose gels, and to map the location of class II subregions on these fragments by probing them with α - and β -chain probes specific for each subregion.

Pulsed-field gel electrophoresis can be used to separate individual yeast and trypanosome chromosomes^{10,19}. To use the technique effectively for mapping genomic regions in higher eukaryotes, we digested the DNA with infrequently-cutting enzymes. In addition to restriction enzymes with 8-base pair (bp) recognition sites, we used restriction enzymes with 6-bp recognition sites that include CG dinucleotides¹⁰. These CG pairs are rare in the human genome²¹. Further advantage in mapping could be gained by the use of partial digestions to identify larger fragments encompassing several subregions.

Several enzymes provide results that establish the linkage between the DR and DQ subregions. *SacII* digests produce two bands of 500 and 450 kb when probed with DR or DQ subregion probes (Fig. 1a). Because both bands hybridize with DR and DQ probes, they probably represent a partial digest. *NotI*, *MluI* and *PvuI* digests result in very large (>675 kb) fragments that hybridize with both DR and DQ probes (Fig. 2). *BssHII* digestion and hybridization with the DQ α - and β -chain probes produce two distinct bands of 200 and 325 kb. The larger of these fragments hybridizes to the DR β -chain probe whereas the smaller does not (Fig. 1a). The DR α -chain probe hybridizes to a distinct 300-kb fragment (Fig. 2). An additional 625-kb band is found (Fig. 1a,b) and in some digests it replaces the 325-kb band that hybridizes with DQ α - DQ β -, and DR β -chain probes (Fig. 1b). This large 625-kb band hybridizes with all DR and DQ subregion probes (Fig. 1a,b) and results from the failure to digest the *BssHII* site between the DR α and DR β loci. This series of *BssHII* digests allows the orientation of the DR α and β loci to be determined, with DR α being the most distal locus of the DR subregion relative to DQ. The maximum size of the region that encompasses the DR and DQ subregions and the genomic sequence between them is defined by the 450-kb *SacII* fragment.

Linkage between the DQ subregion and the locus for DO β was obtained with four different enzymes. The large *MluI* and *NotI* fragments containing the DR and DQ subregions, but not the DP subregion, also hybridize to the DO β probe, establishing the location of this locus at the DR/DQ end of the class II region. A 250-kb *SalI* fragment links DQ and DO as does the 200-kb *BssHII* fragment (Fig. 1b). Neither of these fragments contains DR hybridizing sequences, establishing the DO region as distal to DQ relative to DR. Neither the 325-kb *BssHII* (Fig. 2) nor the 625-kb *BssHII* fragments (Fig. 1b) hybridizes to DO β . The explanation for these data is that a *BssHII* site divides the DQ and DX loci and that the DR β -chains are on the 325- and 625-kb fragments whereas the DO β -chain shares the same 200-kb fragment as the two genes of the DX subregion. These data permit an estimate of the distance between the internal site within the DQ subregion and a site separating the DR β - and α -chains (325 kb). In turn, the maximum size of the portion of the class II region containing DQ and DO is 200 kb (*BssHII*).

Two enzymes show linkage between DO and the DP and DZ loci. A 500-kb *PvuI* fragment contains DO, DZ and DP (Fig. 1c) as do two large partial *ClaI* digest bands of 550 and 475 kb (Fig. 2). Neither of these fragments hybridizes to DR or DQ. The distance between DO and DP must therefore be <475 kb.

Many fragments hybridize to both DP and DZ probes. A *SacII* fragment (200 kb), a *ClaI* fragment (275 kb), a *PvuI* fragment (500 kb), two *BssHII* fragments (300, 375 kb) (Fig. 1c,d), a *SalI* fragment (250 kb), a *PaeR71* fragment (175 kb), a *NarI* fragment (175 kb) and a *NaeI* fragment (200 kb) (Fig. 2), all contain both DP and DZ. Only the *PvuI* (Fig. 1c) and large *ClaI* fragments (Fig. 2) hybridize to DO β in addition to DP and DZ probes. The maximum size of the DP/DZ region is 175 kb, defined by the *PaeR71* fragment. Digests with the enzyme *PaeR71* resolved with a 20-s pulse interval produce a 150-kb band that hybridizes both to DO and DZ. When the DNA is digested with *NotI* and *PaeR71* as a double digest, DZ α hybridizes to a fragment distinct from that hybridizing to DO β , establishing that the original 150-kb *PaeR71* band consisted of two co-migrating bands, one containing the DO β locus and another the DZ loci. A *PaeR71* digest resolved at a 10-s pulse interval confirms that these DO and DZ hybridizing bands are distinct.

The complete linkage between individual subregions can be derived from the fragments illustrated in Fig. 2. Genomic linkage patterns indicate that DQ must be centromeric to DR, and DO centromeric to DQ. DZ and DP are close together and are centromeric to DO. Each of these linkages has been determined with more than one restriction digest.

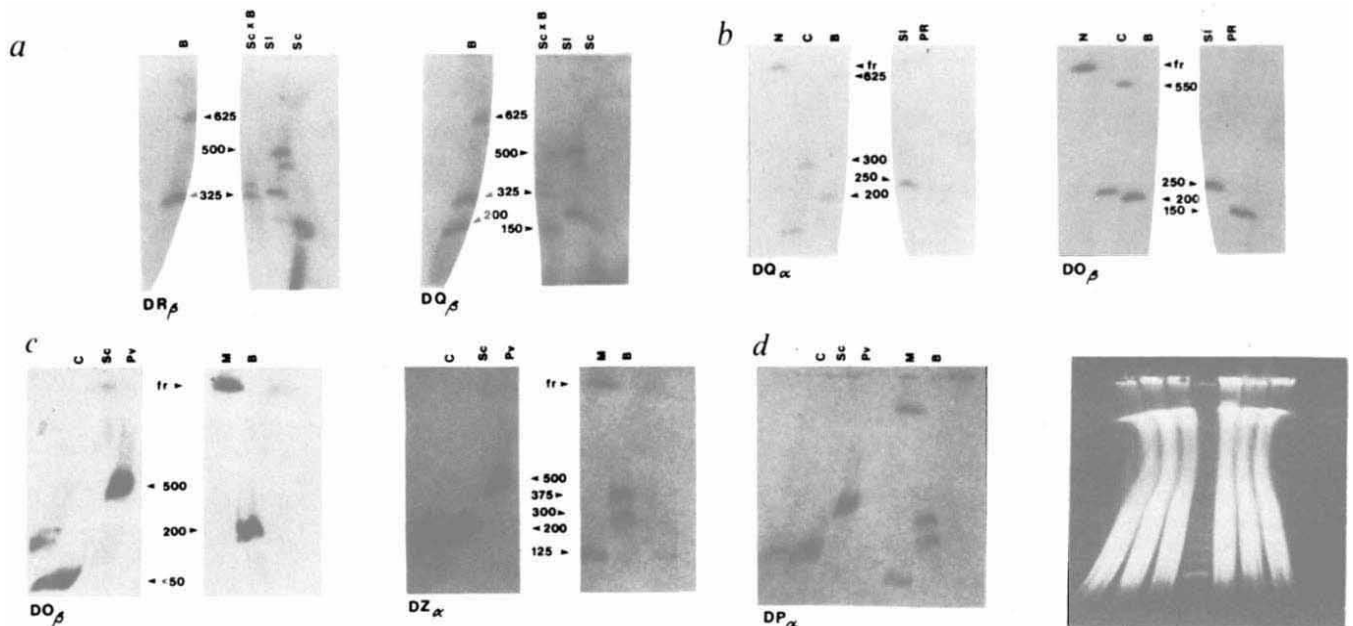


Fig. 1 Pulsed-field gel analysis of genomic DNA from the homozygous consanguineous lymphoblastoid line WT/51 (DR4, Dw4). *a-d*, Hybridization patterns of probes specific for each of the class II subregions. Restriction enzymes: B, *Bss*HII; C, *Cla*I; M, *Mlu*I; N, *Not*I; PR, *Pae*R71; PV, *Pvu*I; Sc, *Sac*II; Sc × B, *Sac*II-*Bss*HII; SI, *Sal*I. All gels were run for 12 h at a 20-s pulse interval. Sizes in kb, determined from ligated lambda phage multimers run simultaneously on each gel (*d*). Fr, large fragments that are not resolved at this pulse interval. *a*, Two bands shared between DR and DQ probes with either B or Sc. An Sc × B double digest results in two bands that hybridize with both DR and DQ probes, one a B band internal to the Sc sites and a second resulting from cleavage of the 625-kb B band with Sc. The SI digest shows distinct DR and DQ bands. *b*, Bands shared between the DQ and DO subregions. SI and B digests each show a shared band hybridizing with both probes. DO hybridizes with a single 200-kb band. The B band at 325 kb is not present on this digest because the large 625-kb fragment was not sufficiently digested to produce that fragment. The N fragment also hybridizes with both DO and DQ probes but was resolved only by longer pulse times (60 s). C and PR bands are distinct for DO and DQ. *c*, Linkage between DO and DZ with PV (500 kb). The C digest on this gel was more complete than *b*, but when the gel in *b* was again probed with DP, the C fragments seen with DO were both present, confirming the DO-DP linkage. *d*, Linkage to DP on the same filter used in *c*. All bands are the same as seen with DZ. An ethidium-stained gel is shown in *d* to illustrate the pattern of the lanes and the lambda phage multimers used for markers.

Methods. Genomic DNA was isolated from cell nuclei prepared by the method of Hieter *et al.*²⁴ and resuspended at $4-6 \times 10^7 \text{ ml}^{-1}$ RSB (10 mM NaCl, 10 mM Tris pH 7.5, 25 mM EDTA). They were then embedded in agarose plugs as described^{19,20}. Briefly, equal volumes of 1.0% low melt agarose in RSB containing $60 \mu\text{g ml}^{-1}$ proteinase K and the nuclei were mixed, transferred to 0.1-ml sample holders and cooled on ice. The embedded nuclei were lysed in RSB with 1.0% SDS at 50 °C for 16–24 h²⁰, washed in 10 mM Tris and 10 mM EDTA, and equilibrated with the appropriate restriction buffer. The genomic DNA was then digested as described²⁵. After digestion, the agarose plugs were melted for 2 min at 65 °C and pipetted directly into the slots of a 1.0% agarose gel in 0.5% Tris-borate-EDTA (TBE)²⁶. The gel was run at 300 V on a pulsed-field gel apparatus²⁶ for 6 or 12 h at 11 °C. Pulse intervals were varied to improve the resolution of the fragments of interest. Small fragments (<300 kb) were electrophoresed at 10-s pulse intervals, medium fragments (300–500 kb) at 20-s pulse times and large fragments (>500 kb) at 30-, 40- and 60-s intervals. Following ethidium bromide staining, the gels were either irradiated at 245 nm for 2 min or treated with acid hydrolysis (0.25M HCl for 30 min). They were then denatured in 0.5 M NaOH and 1.5 M NaCl for 30 min and neutralized in 0.025M $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ at pH 6.5 for 30 min. Southern transfer was performed by standard techniques²⁷ and the DNA fixed by ultraviolet irradiation²⁸ onto nylon membrane (NEN). Pre-hybridization and hybridization were performed by standard techniques²⁷. Probes were labelled to a specific activity of 10^8 – 10^9 c.p.m. μg^{-1} DNA by hexamer priming²⁹ and filters hybridized with 4×10^6 c.p.m. ml^{-1} hybridization buffer. The filters were hybridized for 16 h at 42 °C and washed for 1 h at 25 °C in $2 \times \text{SSC}$ (0.15 M NaCl, 0.015 M $\text{Na}_3\text{citrate}$) and 0.1% SDS and 1 h at 65 °C in $0.1 \times \text{SSC}$ and 0.1% SDS. At these stringencies, the probes are subregion-specific²⁷. After exposing the filters, the probe was removed by washing at 70 °C for 2 h in 5 mM Tris pH 8.0, 0.2 mM Na_2EDTA , 0.05% sodium pyrophosphate, 0.002% polyvinylpyrrolidone (M_r 360,000), 0.002% bovine serum albumin and 0.002% Ficoll (M_r 400,000). The probes used were DRβ complementary DNA clones^{7,30}, DQβ cDNA clones^{7,30}, a DRα cDNA³¹, a DQα cDNA from the DR3 haplotype (J.I.B., unpublished), a DZα genomic probe⁸, a DOβ cDNA probe⁹, DPα probes (H. Erlich, unpublished data and ref. 32) and a DPβ-chain probe³². Individual Southern blots were probed sequentially with α- and β-chain probes to avoid cross-hybridization to residual probe. Where co-migrating bands were suspected, double digests were performed to allow discrimination. Fragments were sized to the nearest 25 kb using lambda phage concatamers.

The closeness of DZ to DP and the low frequency of fragments possessing DO and DZ or DP indicate that the DZα locus is not close to the DOβ locus, thus DZα and DOβ are not a pair of adjacent loci.

Based on the data obtained in this study, the order of the class II subregions can be determined to be [DP DZα]-DOβ-[DX DQ]-DRβ-DRα. The genes encoding DP and DZ have not been linked independently to DO and thus their relative order could not be determined. But recent studies with HLA-deletion mutants show that the DP genes are centromeric to DZα (ref. 13). The genes encoding DX and DQ have been linked independently to DOβ and DRβ but the high degree of similarity between DX and DQ genes preclude their distinction by standard filter hybridization. Two HLA-deletion mutants that have lost DQ and DR genes have retained the DX genes¹³. Taken together, these data show that the DX genes are centromeric to

the DQ genes, and therefore that the order of class II subregions is confirmed as DP-DZα-DOβ-DX-DQ-DRβ-DRα.

The map order has evolutionary implications. Sequence homology indicates that DQ molecules are analogous to the I-A molecules in the mouse and that DR molecules are analogous to the products of the murine I-E loci. Aβ₂ and DOβ are also thought to have a common origin, and DPβ is most similar to Aβ₃ (ref. 11). The map order of these homologous loci in the mouse correlates directly with the map order in man. The size of the human class II region cannot be established precisely by our results, as we do not know how far outside the complex the distal restriction sites lie. We can conclude on the basis of the *Cla*I digests that the region could be as large as 1,100 kb. Three different *Cla*I fragments, each hybridizing to different subregion probes, can be defined and total 1,100 kb. Addition of the three non-overlapping *Bss*HII fragments and the distance between

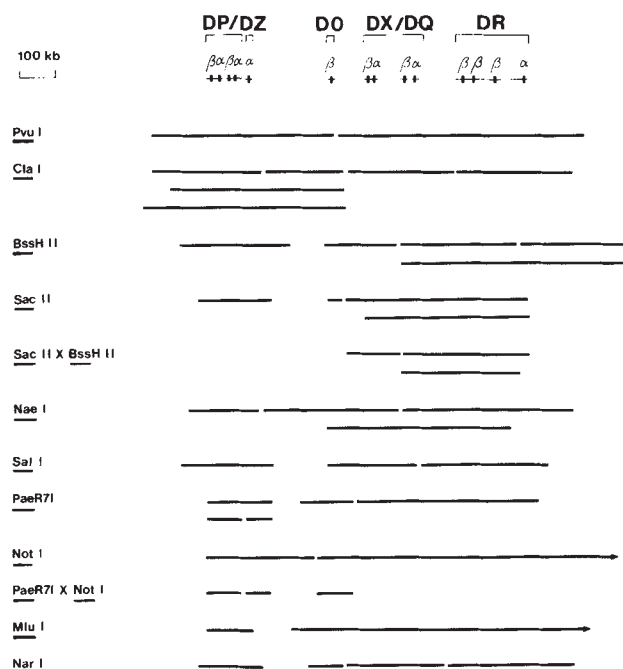


Fig. 2 Map of the linkage of the different subregions of the class II region of the human MHC. Fragments obtained with each enzyme are illustrated to scale below the subregions with which hybridization has been demonstrated. The only exception is the 500-kb *NaeI* fragment, which was included because it confirms the location of *DRα*, but was not probed with the *DOβ*-chain probe. Sizes of fragments were determined based on gels run at appropriate pulse intervals. Only restriction fragments that were contributory to linkage are shown. Restriction sites are not mapped precisely in relation to each other unless indicated by double digest. Additional lines for a particular enzyme indicate contributory fragments obtained from different digests. Because of the proximity of *DZ* to *DP*, their order relative to each other is uncertain. With the exception of *DRα*, the order of the α and β loci within a subregion has not been determined.

the *BssHII* site within the *DR* subregion of *DR3* haplotype to the end of the *DRα* locus¹⁶ again results in a total estimate of the size of the region as 1,100 kb. The similarity of these two estimates indicates that the human class II region is significantly larger than that of the mouse.

No recombination between *DQ* and *DR* genes has yet been reported and alleles of these genes are in very strong linkage disequilibrium. In contrast, a high recombination frequency of ~1–3% occurs between *DP* genes and *DQ-DR* genes²². Furthermore, there is very little linkage disequilibrium between *DP* and *DR* alleles²³. These observations suggest that either the *DP* subregion is located some distance centromeric to the *DQ-DR* subregions, or that a recombination 'hot spot' exists between *DP* and *DQ-DR*. Our results exclude the first possibility as the distance between *DP* and *DQ* is similar to the distance separating *DQ* and *DR*. Pulsed-field electrophoresis mapping can now be used to locate these recombination points.

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Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain

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Gelsolin is representative of a class of actin-modulating proteins found in lower eukaryotes to mammals, which sever actin filaments¹. Gelsolin found in the cytoplasm of cells is functionally similar to a mammalian plasma protein of similar size, originally called ADF or brevins. Human plasma and rabbit macrophage gelsolins differ by the presence of a 25-amino-acid residue extension on plasma gelsolin which appears to account for the difference in relative molecular mass (*M_r*) between the proteins as assessed by SDS-polyacrylamide gel electrophoresis (PAGE), 93,000 (93K) and 90K, respectively². Here we report the isolation of full-length human plasma gelsolin complementary DNA clones from a HepG2 library. The inferred amino-acid sequence reveals the presence of a signal peptide, a long tandem repeat that matches the actin-binding domains of gelsolin, a tetrapeptide present in actin and extended regions of identical sequence with rabbit macrophage gelsolin. Southern blot analysis indicates that a single gene in the haploid genome encodes both protein forms.

The human hepatoma cell line HepG2 makes a large amount of plasma gelsolin². We prepared a cDNA library from RNA

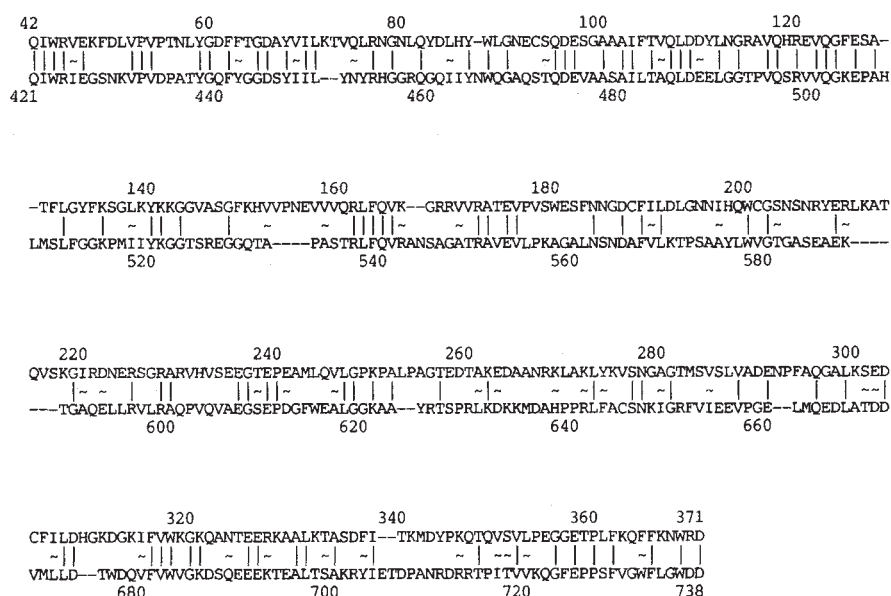
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vector DNA, packaged *in vitro* and plated on *Escherichia coli* host strain C600HFL. The resulting library contained $\sim 2 \times 10^6$ independent recombinant clones. Two redundant oligonucleotides were synthesized (Applied Biosystems), corresponding to residues 410–416 and 517–522, and labelled with ^{32}P using T4 polynucleotide kinase and ^{32}P -dATP. Two λ GT10 clones, G42 and G43, were identified by oligonucleotide hybridization and subcloned into the *Eco*RI site of M13mp18. A series of overlapping subclones was generated²¹ from complementary M13mp18 clones and sequenced by the dideoxy method²². The two clones were identical for 2,149 bases (460–2,608), with the derived amino-acid sequence matching 6 of the peptide fragments of gelsolin. Both clones seemed to contain non-gelsolin sequences at their 5' ends, so the cDNA library was rescreened with a ^{32}P -labelled single-stranded M13 probe made from a 408-bp *Sau*3A fragment of G42 (bases 1,646–2,054) subcloned into M13mp19. Six new clones were identified, subcloned into M13mp18 and sequenced. One new clone, GG2, contained sequences encoding bases 23–2,608, including peptide sequences *a-d*. Comparison of the GG2 sequence with the 5' sequences of G42 and G43 showed no relationship, confirming that these regions of G42 and G43 were non-gelsolin sequences. Further library screening using a ^{32}P -labelled single-stranded M13 probe derived from the 201-bp *Eco*RI-*Bgl*III 5' fragment of GG2 (bases 23–224) subcloned into M13mp19 yielded clone GM1 which encodes 23 additional residues beyond GG2 and contains an initiator methionine. None of the 7 clones obtained in secondary rounds of screening, including GG2 and GM1, contain any unrelated sequence as assessed by sequencing of both ends of each clone as well as restriction mapping of the cDNA inserts.

[illegible]

Fig. 2 The 330-residue repeated sequence. Predicted gelsolin sequence is shown from residues 42-371 and 421-738, respectively. |, Identical residues; ~, homologous residues. A, I, L, V, Q, N, F, Y, W, E, D, H, R, K, and S, T, homologous residues for our analysis. The alignment was generated using the ALIGN program of BIONET.



isolated from confluent HepG2 cells and identified gelsolin clones by screening with labelled oligonucleotide probes. Rescreening with the original isolates led to the isolation of full-length plasma gelsolin cDNA clones. The cDNA sequence and derived protein sequence are shown in Fig. 1. The longest cDNA clone included 95 bases upstream from the codon for the first amino-acid residue of mature plasma gelsolin. Within this region is a single methionine codon, beginning at base 15, with surrounding bases matching the consensus sequence for initiation (CCA/GCCAUGG)³. With initiation of translation at this codon, the resulting protein is in-frame with the downstream sequence of mature plasma gelsolin. The 27-amino-acid residue extension has the typical features of a signal peptide⁴. The predicted amino-acid sequence is 755 residues long and contains all 10 of the amino-terminal amino-acid sequences determined from gelsolin peptide fragments with only 1 mismatch out of 187 residues. This includes two sequences derived from rabbit macrophage gelsolin (Fig. 1, peptide c, residues 26-64) and a peptide fragment of macrophage gelsolin (Fig. 1, peptide d, 66-85). The calculated M_r for gelsolin is 83K, consistent with estimates based on hydrodynamic measurements (68-93K)^{5,6} and 10% smaller than values based on relative mobility during SDS-PAGE⁵. The predicted sequence does not contain the consensus sequences for N-linked glycosylation, Asn-X-Ser or Asn-X-Thr, consistent with previous observations² that plasma gelsolin is not glycosylated.

There is a strong tandem repeat in the amino- and carboxy-terminal halves of plasma gelsolin (Fig. 2, residues 42-371, 421-738). This repeated sequence is 318 amino-acid residues long with 9 short gaps (≤ 7 residues) and 109 (34.3%) identical residues out of 151 (47.5%) homologous residues. In addition, comparison of the hydrophobicity/hydrophilicity profiles of the two sequences indicates a very similar pattern (data not shown). This sequence repeat is of interest in comparison with data available on the functional domains of gelsolin. Gelsolin can be cleaved by chymotrypsin into a carboxy- and an amino-terminal half^{7,8}. We have shown⁷ that the amino-terminal half (CT45) contains a Ca^{2+} -insensitive actin-filament-severing domain and have localized this activity to a 15K subfragment close to the amino terminus of gelsolin (CT15)⁹. We also found that the carboxy-terminal half (CT47) contains a Ca^{2+} -sensitive actin-binding domain⁹, based on inhibition of pyrenyl-actin assembly and binding to actin-Sepharose beads, which does not sever actin filaments. Because intact gelsolin requires Ca^{2+}

to bind actin and sever actin filaments, these data suggest that the carboxy-terminal half imposes a Ca^{2+} requirement on the amino-terminal actin-severing domain. A similar model for the coordinate interaction between the two halves of the molecule is proposed by Bryan and Hwo⁸, except that they find the amino-terminal half has two actin-binding sites and the carboxy-terminal half has none. Based on the relative locations of CT15, CT31 and CT47 (Fig. 1, peptides b, e and g, respectively) within the gelsolin sequence and the quantitative yield of these peptides from gelsolin by digestion with chymotrypsin⁹, we deduce that CT15 is Ser 24-Phe 149 and CT47 is Ala 407-Ala 755. The division into amino- and carboxy-terminal actin-binding domains in our model therefore agrees with the observed internal repeat in the amino-acid sequence of gelsolin. Although CT47 is regulated by Ca^{2+} , its predicted protein sequence does not reveal any site conforming to the E-F-hand paradigm¹⁰ characteristic of several calmodulin-like Ca^{2+} -binding proteins. There is a partial E-F-hand match in the amino-terminal half (residues 339-366), but the significance is uncertain.

Analysis of the protein sequence for further similarities within gelsolin using the SEQHP program¹¹ (based on the similarity matrix of Dayhoff *et al.*¹²) reveals a more elaborate repetitive structure. There are 6 regions of homologous sequence (85-196, 197-314, 315-397, 462-570, 571-675, 676-738) in a tandem alignment with a gap of 65 residues (398-461) separating the third and fourth repeats. This weak homology was confirmed using the SEQDP program¹¹. The sixfold repetitive structure does not correlate with known functional data on gelsolin, and

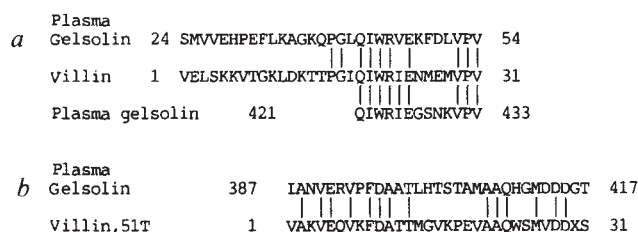


Fig. 3 Sequence comparison between gelsolin and villin. a, amino-terminal sequences; b, middle sequences; 51T, the carboxy-terminal half of villin¹⁶; starting and ending residues are indicated. |, Identical residues. x, unknown residue.

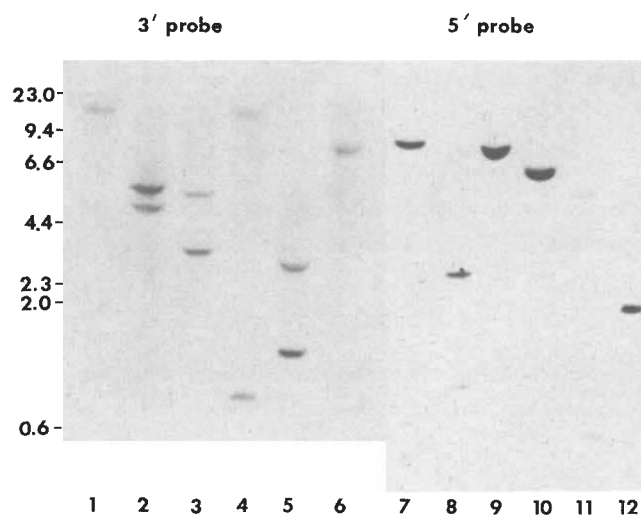


Fig. 4 Southern blot analysis of human genomic DNA using the plasma gelsolin cDNA. Lanes 1–6, autoradiograph of a Southern blot of human genomic DNA digested with enzymes *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III, *Pst*I and *Sac*I, respectively, and probed with the 32 P-labelled 3' cDNA fragment (bases 2,078–2,603); lanes 7–12, blot derived from DNA digested with the same enzymes as lanes 1–6 and probed with the 32 P-labelled 5' cDNA fragment (bases 2,078–2,603). *M*, markers are indicated (kb).

Methods. Human genomic DNA isolation, restriction enzyme digestion, agarose gel electrophoresis and transfer to GeneScreen Plus were all according to standard methods. Prehybridization was for 1 h at 65 °C in 1% SDS, 1 M NaCl, 10% dextran sulphate, 0.25 mg ml⁻¹ denatured salmon sperm DNA; hybridization was in the same solution for 12–16 h at 65 °C. Probes were prepared by digestion of subcloned cDNA insert and gel purification followed by labelling using 50 μ Ci [α - 32 P]dCTP by the random hexanucleotide method²³ to a specific activity of 0.5–1.0 $\times 10^9$ c.p.m. μ g⁻¹. Final wash, 30 mM NaCl, 3 mM Na citrate pH 7.0, 0.05% SDS at 65 °C for 30 min. Autoradiographs were exposed with an enhancing screen at –70 °C for 6–24 h.

possibly reflects the genesis of the protein through ancestral repeated gene duplications.

We observe a striking homology between gelsolin and villin, a protein of vertebrate brush border microvilli that is functionally similar to gelsolin¹³. Like gelsolin, villin is a Ca²⁺-regulated, actin-filament-severing, nucleating and end-blocking protein^{14,15}, although in contrast to gelsolin, villin crosslinks actin filaments into parallel bundles when Ca²⁺ < 10⁻⁶ M. This crosslinking activity is abolished by removal of an 8.5K carboxy-terminal fragment (the headpiece)¹⁴ and the residual peptide (the core) has all the other activities. Figure 3a demonstrates the previously reported homology¹⁶ between the amino-terminal sequences of human plasma gelsolin and chicken villin, as well as the comparison between these sequences and the second tandem repeat in gelsolin. There is also strong homology between Ile 387–Thr 417 of plasma gelsolin and the carboxy-terminal half of villin (51T) (14 out of 30 identical residues without gaps; Fig. 3b). The 76-residue sequence of chicken villin headpiece¹⁷ shares no similarity with any portion of gelsolin. Thus, the functional similarities between gelsolin and villin recapitulate primary sequence homologies between the two proteins, suggesting that the proteins belong to the same family and share a common ancestor, and that other actin-filament-severing proteins from invertebrates may share homology with gelsolin and villin. In contrast, there is no significant homology between gelsolin and the few other actin-binding proteins for which sequence data have been reported. The gelsolin sequence is also distinct from that of actin, except for the presence of the tetrapeptide Asp-Glu-Ser-Gly (residues 96–99) in the severing domain, which is also found near the carboxy terminus (residues 363–366) of mammalian cytoplasmic actin, a region thought to participate in the actin-actin contact between adjacent actins in the filament¹⁸. We suggest that this peptide sequence in

gelsolin is part of the actin-severing domain.

Gelsolin is unique among mammalian proteins in that one form is produced for intrinsic intracellular use and another is made specifically for secretion². The two proteins are functionally similar and two amino-acid sequences derived from rabbit macrophage gelsolin are the same as the plasma gelsolin sequence (Fig. 1, peptides *c* and *d*). To determine if these two proteins are encoded by a single gene, we performed Southern blot analyses of restriction enzyme-digested human genomic DNA (Fig. 4). We observed multiple bands when DNA digested with different restriction enzymes was probed with the full-length gelsolin cDNA (data not shown). However, when similar blots were probed with a 5' fragment (bases 1–390) or a 3' fragment (bases 2,078–2,603) of the clone, there were single bands for several different enzymes, suggesting that a single gene encodes the two proteins. The possibility of two tightly clustered duplicated genes in a single restriction fragment is excluded by the fact that the largest bands seen in two instances (Fig. 4, lanes 4, 12) are <3 kilobases (kb) long and therefore too small to harbour two gelsolin genes. These observations strongly suggest that a single gene is present in the haploid human genome to encode both plasma and cytoplasmic gelsolin.

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Bending of promoter DNA on binding of heat shock transcription factor

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The *Drosophila* heat-shock transcription factor (HSTF) has been shown to bind to three domains of the heat shock protein 70 gene (*hsp 70*) control region^{1,2}. The most critical of these for transcriptional activation appears to be the one closest to the TATA-homology region^{2,4}. This domain, spanning sequences from -40 to -95, consists of two contiguous HSTF binding sites (sites 1 and 2) that are occupied in a cooperative manner² (see Fig. 1). Recent alkylation interference and protection studies suggest a conformational change occurs in the protein-DNA complex at site 1 upon sequential HSTF binding at site 2 (ref. 5). We report here that HSTF binding to a single site or to both contiguous sites results in the introduction of a specific DNA bend within this domain of the *hsp 70* promoter.

It is well known that proteins can alter the conformation of DNA. Examples of stable DNA bending include nucleosomes in eukaryotes^{6,7} and possibly DNA binding protein II complexes in prokaryotes⁸. More recently, DNA bending induced by site-specific DNA binding proteins has also been documented. Rosenberg *et al.* have obtained co-crystals of the restriction enzyme *EcoRI* with its recognition site that diffract to high resolution, revealing dramatic DNA kinks⁹. Prior to these studies, DNA bending had been postulated in an effort to maximize the proposed crystal complementarity of the structures of *cro*^{10,11} and CAP¹² to their target sequences in B-form DNA. Wu and Crothers provided evidence supporting this predicted DNA bending for CAP bound at the *lac* operon using a protein-DNA complex gel assay¹³. This inferred bending was confirmed by direct electron microscopic visualization of CAP bound to the *lac* promoter element¹⁴. DNA bending has also been demonstrated by electron microscopy of size fractionated transcription complexes from HeLa cell extracts where a striking bend has been mapped near the site of initiation on a *Xenopus laevis* vitellogenin gene¹⁵. The functional significance of these DNA structural changes remains to be demonstrated. In this report we show that DNA bending occurs upon site-specific DNA binding of a transcriptional regulator in eukaryotes—HSTF of *Drosophila*.

The HSTF, purified from nuclei of heat-shocked *Drosophila* Kc cells, has been shown to be a positive activator of *hsp 70* transcription but not for a control actin gene in reconstituted *in vitro* transcription studies¹. DNase I footprinting experiments demonstrate that the HSTF also binds to the 5' control regions of many other *Drosophila* heat-shock genes¹⁶. Within each binding site, the heat-shock consensus sequence C--GAA--TTC--G can be found^{2,3}. *In vivo*^{3,4} and *in vitro*² transcription studies implicated the contiguous TATA-homology proximal to binding sites 1 and 2 as the most critical for transcriptional activation. Biochemical analysis has revealed that the HSTF binds cooperatively to these sites with a 10–20-fold higher intrinsic affinity for site 1 than for site 2 (ref. 2). Using a protein-DNA complex gel separation technique it is possible to resolve species consisting of HSTF bound to site 1 (complex A) and HSTF bound to both sites 1 and 2 (complexes B and B*)². The precise HSTF stoichiometries in the three complexes are not known. We have previously postulated from a contact point symmetry argument that complex A has a HSTF dimer bound at site 1 while in complex B dimers occupy both sites⁵. Recent dimethyl sulphate interference and protection studies suggest complex B* consists of a dimer bound at site 1 and a monomer bound at the proximal

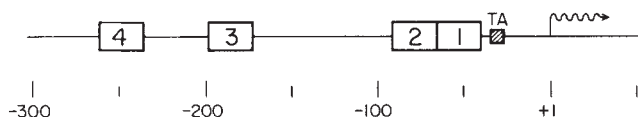


Fig. 1 The *hsp 70* promoter structure showing the positions of the four HSTF binding sites, the TATA-homology region (TA), and the start point of transcription (wavy arrow).

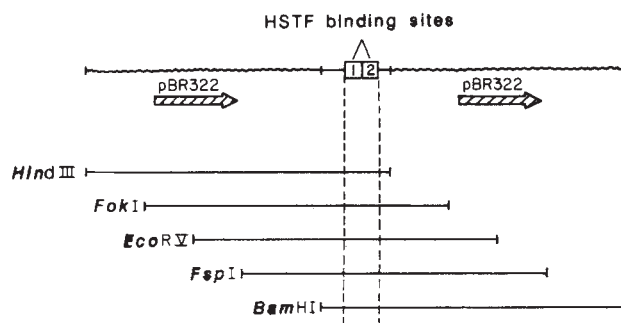


Fig. 2 Restriction fragments harbouring HSTF binding sites 1 and 2 of the *hsp 70* promoter used to investigate DNA bending. The 346-base pair (bp) direct repeats of pBR322 (wavy lines) flank a 101-bp stretch of *Drosophila* DNA containing the HSTF binding domain. Shown below are the fragments generated upon cleavage with the indicated restriction enzymes. The position of the binding sites relative to the ends of each fragment is shown with the dashed lines.

Methods. The clone aDm3111 was constructed by a two-step procedure. Plasmid pBR322 was restricted with enzymes *Bam*HI and *Hind*III and the 346 bp fragment was isolated by gel electrophoresis. This fragment was subsequently treated with phosphatase. A 10-fold molar excess of this DNA was ligated to 1 µg of the 101-bp gel-purified *Bam*HI/*Hind*III insert of plasmid aDm3110 containing HSTF binding sites 1 and 2. The ligation mixture was applied to an agarose gel and the 793-bp product was isolated. This fragment was subsequently inserted into a *Bam*HI/*Hind*III restricted pUC9 vector.

half of site 2 (D.S., unpublished results). Support for this notion comes from the observation that complexes A and B* can be shifted to complex B at higher HSTF concentrations (Fig. 3a and unpublished results). Similar experiments demonstrate that the HSTF can also form at least three distinct complexes with the analogous promoter region of the *Drosophila* *hsp 83* gene (D.S., manuscript in preparation).

Recently, the protein-DNA complex gel separation technique was used to determine the critical contacts HSTF makes with its target sequence⁵. Interestingly, the contacts within site 1 differ when complexes A and B are analysed suggesting that a conformational change occurs to the HSTF-site 1 complex upon sequential HSTF binding at site 2. Thus, we wished to ask whether changes in DNA structure played a significant role in this apparent conformational change.

As discussed by Wu and Crothers¹³, the mobility of identically sized DNA fragments containing a specific DNA bend is dependent upon the position of the bend relative to the ends of the fragment. In accordance with gel electrophoresis theory^{17,18}, when a bend is located near the end of the molecule it will migrate faster than a molecule with the bend centrally located. We constructed the clone aDm 3111, drawn in Fig. 2, by inserting a segment of the *hsp 70* promoter harbouring HSTF binding sites 1 and 2 between direct repeats of a pBR322 restriction fragment. Digestion with the indicated enzymes yields fragments of identical size and base pair composition with variant relative

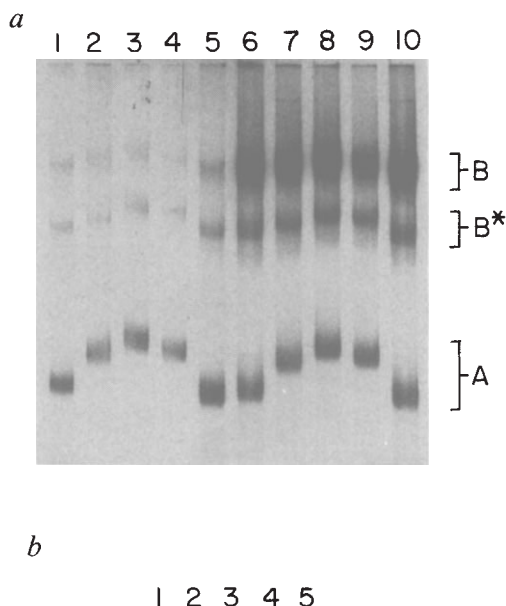


Fig. 3 *a*, HSTF-DNA complex gel analysis. Autoradiograph of a protein-DNA complex gel with the positions of complexes A, B and B* indicated at the right. Complexes formed with the labelled restriction fragments of enzymes *Hind*III (lanes 1 and 6), *Fok*I (lanes 2 and 7), *Eco*RV (lanes 3 and 8), *Fsp*I (lanes 4 and 9), and *Bam*HI (lanes 5 and 10) are shown. Lanes 1-5 contained 0.20 μ g HSTF in the initial binding reaction while lanes 6-10 contained 0.60 μ g of HSTF. *b*, Gel analysis of purified fragments in the absence of HSTF. Lanes 1-5 contain *Hind*III, *Fok*I, *Eco*RV, *Fsp*I, and *Bam*HI restriction fragments, respectively.

Methods. Plasmid aDM3111 was restricted with the indicated enzymes, treated with phosphatase, and labelled at the 5' ends with [γ - 32 P]ATP and polynucleotide kinase. The 447-bp fragments of each digest were gel-purified by elution onto DE-81 membranes. HSTF was purified from heat-shocked *Drosophila* Kc cell nuclei as previously described. Approximately 0.005-0.01 pmol of the DNA fragments (10,000 c.p.m.) were incubated with 0.2 or 0.6 μ g HSTF in the standard binding conditions consisting of 20 mM HEPES (pH 7.6), 5 mM MgCl₂, 60 mM KCl, 4% Ficoll, 3% glycerol, 0.03 mM EDTA, and 50 μ g ml⁻¹ pBR322 plasmid DNA restricted with *Hin*FI. Following incubation at 22 °C for 5 min the samples were chilled on ice and applied directly to a vertical 2% agarose gel. Electrophoresis was carried out at 400 V for 4-6 h at 4 °C in 25 mM Tris, 25 mM boric acid and 1 mM EDTA. The gels were dried and exposed for autoradiography. In panel *b* electrophoresis was for 2 h only to ensure the unbound DNA fragments remained on the gel.

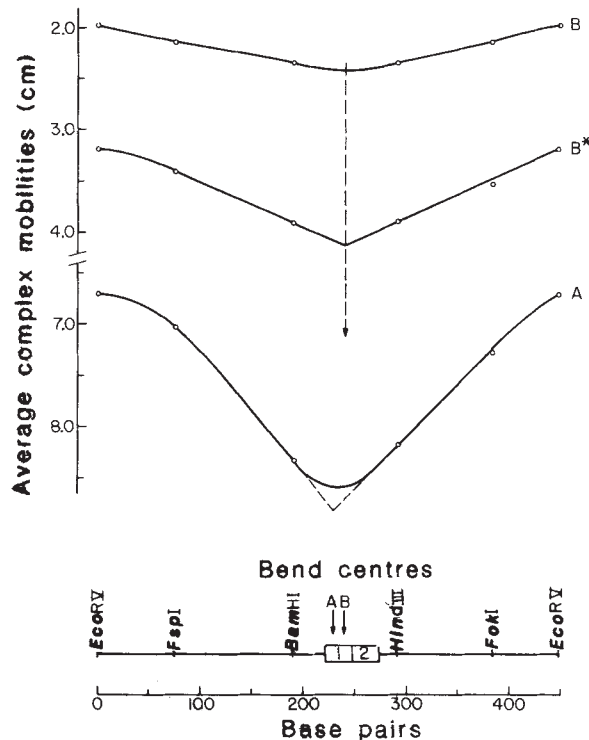


Fig. 4 Mobility plot of the HSTF-DNA complexes. Shown is a plot of the mobility of complexes A, B, and B* for each fragment used in this study against the map positions of the corresponding restriction sites. The bend centre of complex A was mapped by extrapolating the linear portions of the curve to a point on the DNA fragment. Complex B and B* bend centres were assigned as the midpoint of the identically migrating *Bam*HI and *Hind*III restriction fragments. The mobility data shown represent an average of five separate experiments.

positions of the binding loci. These five restriction fragments were end-labelled, gel-purified, and incubated with HSTF purified from heat-shocked *Drosophila* Kc cells. The binding conditions are essentially the same as those described previously for DNase I footprinting studies² with the addition of 4% Ficoll to facilitate gel loading. After a brief incubation at 22 °C the reactions are chilled and applied directly to an agarose gel at 4 °C. Following electrophoresis, the gels are dried and exposed for autoradiography. Figure 3*a* shows the results of such an experiment. Lanes 1-5 have 0.2 μ g HSTF per binding reaction while lanes 6-10 contain 0.6 μ g HSTF. As mentioned previously, complex A has HSTF bound to site 1 whereas complexes B and B* have HSTF bound to both sites (see below). The DNA fragments produced by *Hind*III (lanes 1 and 6) and *Bam*HI (lanes 5 and 10) cleavage, have HSTF binding sites close to the ends of the fragment. The DNA fragments produced by *Fok*I, *Eco*RV, and *Fsp*I harbour more centrally located HSTF binding

sites. When the HSTF binds this set of fragments, complexes formed at terminal binding loci migrate faster than those with more centrally located sites indicative of a protein-induced site-specific DNA bend (Fig. 3*a*). It is apparent from these data that bending occurs in all three complexes (A, B and B*). In Fig. 3*b*, the DNA alone is electrophoresed to show that the fragments themselves show no observable bending. The unbound DNA migrates about twice as fast as complex A and is normally electrophoresed off the gel. During the course of this investigation a variety of gel systems, including acrylamide and acrylamide/agarose composite gels, were utilized. In each case the characteristic anomalous migration of the HSTF-DNA complexes was evident whereas the mobilities of the unbound DNA fragments were identical. The extreme size of these complexes, calculated to be minimum of 500 kilodaltons for complex B, dictates agarose gels to be the system of choice for this study.

A plot of the average mobility data from five experiments versus a map of the corresponding restriction sites is shown in Fig. 4. Note that each fragment can be viewed as originating from the enzymatic linearization of a circular version of the *Eco*RV fragment shown. Extrapolation of the linear portions of the complex A curve map the bend centre to site 1 as expected. Accurate mobility data for complexes B and B* data are more difficult to obtain due to their extremely slow relative migration in this gel system. It is clear, however, that the *Bam*HI and *Hind*III restriction fragments migrate identically in complexes B and B* in contrast to complex A (Fig. 3*a*; compare lanes 5 and 6). Even when electrophoresis is carried out for a significantly longer period of time, the mobilities of these two fragments in complexes B and B* are indistinguishable (data

not shown). Therefore, the bend centre was assigned as the midpoint of these restriction sites (*Bam*HI and *Hind*III), and is shifted towards site 2 relative to the complex A bend centre. The observation that the bend centres in both complexes appear to be skewed towards the direction of transcription is interesting in that a similar asymmetric bend is found when the CAP dimer is bound to the *lac* promoter¹³. It is possible that asymmetric bend centres may in fact serve a biological role. It should be noted that this study uses larger DNA restriction fragments than those used in the CAP bending study and thus we expect a correspondingly larger error in mapping the bending loci. We estimate this error to be ± 5 –7 base pairs.

With the data available one can only speculate on the possible significance that a bend at the promoter might have. von Hippel and co-workers^{19,20} have postulated that sequence-specific DNA binding proteins scan the template for their target sequence by a sliding mechanism. If this were the case a bend may somehow signal a sliding protein (perhaps RNA polymerase II) to slow or stop at that locus, facilitating a stable productive interaction. It is also conceivable that a DNA bend may bring the bound protein molecules into proximity with other regulatory factors enabling required protein-protein interactions to occur. That such interactions are important in gene transcription has been demonstrated by Ptashne and collaborators^{21,22} who have shown that maximal activation of the λ promoter P_{RM} almost certainly involves protein-protein contacts between the phage repressor bound at *OR2* and RNA polymerase. Additionally, cooperative binding of λ repressor dimers bound at operators separated by five or six turns of the helix²³ is thought to occur by a DNA bending mechanism. Transcriptional repression of the *araBAD* operon of *Escherichia coli* may actually involve interactions of repressor molecules bound to operators 150 base pairs apart²⁴. Recently, Takahashi *et al.*²⁵ provided evidence that the spatial distribution of the simian virus 40 promoter elements is critical, suggesting that eukaryotic gene activation may depend upon the three-dimensional topology of transcription complexes. An alternative and less intriguing prospect, however, is that DNA bending could serve no regulatory function and may simply represent the most energetically favourable DNA configuration for interaction with a given protein.

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Cloning and sequence analysis of cDNA for bovine carboxypeptidase E

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Carboxypeptidase E (enkephalin convertase) was first identified as the carboxypeptidase B-like enzyme involved in the biosynthesis of enkephalin in bovine adrenal chromaffin granules¹. A similar enzyme is present in many brain regions^{1,2} and in purified secretory granules from rat pituitary³ and rat insulinoma⁴. Within the secretory granules, carboxypeptidase E (CPE) activity is found in both a soluble and a membrane-bound form¹, which differ slightly in relative molecular mass (M_r)⁵. Here, to investigate whether the CPE activities in the various tissues are produced from a single gene, purified CPE was partially sequenced and oligonucleotide probes were used to isolate a clone encoding CPE from a bovine pituitary complementary DNA library. This cDNA hybridizes to bovine pituitary poly(A)⁺ RNAs of approximately 3.3, 2.6 and 2.1 kilobases (kb), with the 3.3-kb messenger RNA the predominant species. The predicted amino-acid sequence of the cDNA clone contains the partially determined sequences of CPE, several pairs of basic amino acids and displays some homology with both carboxypeptidases A and B. Restriction analysis of bovine genomic DNA suggests only one gene for CPE. This is consistent with a broad role for CPE in the biosynthesis of many neuropeptides.

CPE was purified to homogeneity from bovine pituitaries as described previously⁶. The amino-acid sequence of the amino-terminus of CPE was determined by gas-liquid sequencing⁷ of 200 μ g of purified enzyme. Both the soluble and the membrane-bound forms of CPE were found to have the same amino-terminal amino-acid sequence. To obtain further sequence information, 500 μ g of the soluble forms of CPE was digested with *Staphylococcus aureus* protease, fraction V-8 (Sigma), in 0.1 M NH_4HCO_3 . Peptide fragments were then purified on a HPLC reverse-phase C_{18} column⁷, and the amino-acid sequence of several purified fragments was determined (Fig. 1 legend).

Three pools of oligonucleotides complementary to the predicted CPE mRNA sequence were synthesized using the phosphoramidite method⁸. The oligonucleotide pools consisted of all possible mRNA sequences complementary to the regions indicated in Fig. 1. The amino-terminal probe pool (5'-ATNCC⁺TC⁺TC⁺TGNGG-3'), the G-1 fragment pool (5'-AC⁺TC⁺ATG⁺TC⁺AT-3') and the L-1 fragment pool (5'-TC⁺AA⁺TCNACNC⁺NAC-3') were labelled with ³²P using polynucleotide kinase and [γ -³²P]ATP. These probes were then used to screen a bovine pituitary cDNA library generated using the Okayama-Berg method⁹. A total of 10⁶ colonies were screened by conventional methods¹⁰, and one colony was found which hybridized with all three probes. Because this library has been amplified, the abundance of cDNA clones encoding CPE does not reflect the abundance of the CPE mRNA in bovine pituitary.

Plasmid DNA was isolated from this positive colony, and the cDNA insert (1,485 base pairs; bp) was isolated and sequenced (Fig. 1). The sequence of the 5' end of the cDNA coincides with that of the amino-terminal of the active enzyme, and no information concerning a precursor form or signal peptide could be obtained from this partial cDNA clone. The determined se-

Hybridization of size-fractionated poly(A)⁺ RNA from the bovine pituitary with ³²P-labelled CPE cDNA (Northern blots) detects three species of mRNA (Fig. 2a). Similar results are found with poly(A)⁺ RNA isolated from bovine adrenal medulla and various brain regions (data not shown). The most abundant species migrates with an apparent size of 3,300 bp. Other mRNA species corresponding to 2,600 and 2,100 bp also hybridize to the cDNA under fairly stringent conditions. It is likely that these differently sized mRNAs are a result of variations in the 3'-untranslated tail due to alternative polyadenylation sites. The

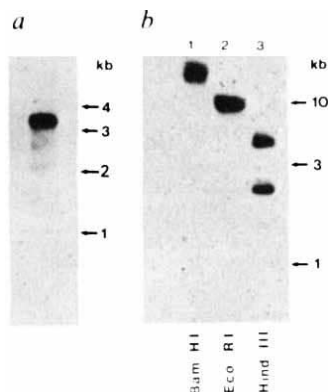


Fig. 2 Northern blot analysis of bovine anterior pituitary poly(A) RNA (a) and Southern blot analysis of bovine genomic DNA (b). Lanes 1–3 in b show the results of digestion with different restriction enzymes.

Methods. a, Poly(A) RNA (20 µg) isolated from bovine anterior pituitary was electrophoresed in a 1.2% agarose gel with formaldehyde¹⁰. The RNA was transferred to nitrocellulose¹⁰ and hybridized with 1.4×10^6 c.p.m. of a 32 P-labelled 1.3-kb CPE cDNA fragment (*Pvu*II 307 to the 3' end of the shorter cDNA clone). Hybridization was overnight at 42 °C in 5×SSC, 0.1% Sarkosyl, 0.1% pyrophosphate, 1×Denhardt's, 250 µg ml⁻¹ salmon sperm DNA and 50% formamide. After washing in 2×SSC at 42 °C, the blot was exposed to film overnight at -70 °C using a Dupont lighting plus intensifying screen. A 1-kb ladder (Bethesda Research Laboratories) was used as DNA size standards. b, Bovine genomic DNA (20 µg) was digested with *Bam*HI, *Eco*RI or *Hind*III, electrophoresed on a 0.8% agarose gel and transferred to nitrocellulose¹⁰. A 327-bp CPE cDNA fragment (*Pvu*II 307 to *Hae*III 634) was labelled with 32 P by nick translation and hybridized to the genomic blot overnight at 55 °C. The hybridization buffer was similar to the buffer used for the Northern blot, except that the formamide was omitted. A 1-kb ladder was also used as a DNA size standard.

3' end of the cDNA clone sequenced does not contain the typical AATAAA signal for polyadenylation. Instead, an AAATATAA is present 22 bp before the poly(A) tail which is not a common polyadenylation site.

Rescreening the bovine pituitary cDNA library with 32 P-labelled CPE cDNA reveals another clone which encodes CPE but contains an additional 1.2 kb at the 3' end. The partial sequence of this clone (Fig. 1) is identical to the sequence of the original clone where the two clones overlap. However, in place of the poly(A) tail of the original clone, the longer clone continues for 1,200 bp before the poly(A) tail is found. Preceding this poly(A) tail is the typical polyadenylation signal (AATAAA). Thus, it seems likely that the original clone rep-

resents the 2.1-kb mRNA, while the longer clone represents the more abundant 3.3-kb mRNA.

Restriction analysis of bovine genomic DNA (Southern blots) reveals a single gene for CPE (Fig. 2b). When genomic DNA is digested with either *Bam*HI or *Eco*RI, size fractionated and blotted onto nitrocellulose, 32 P-labelled CPE cDNA hybridizes to a single band (Fig. 2b, lanes 1, 2). The cDNA fragment used as a probe contains a *Hind*III site, and so when genomic DNA is digested with *Hind*III, two major bands hybridize to the probe (Fig. 2b, lane 3). A third band is also seen, which suggests that a *Hind*III-containing intron is present in this region. Analysis of Southern blots with other cDNA fragments suggests the presence of many introns (>5) within the region coding for CPE (data not shown). However, it is also possible that the third band represents another gene with homology to CPE. A search of the National Institutes of Health GenBank Database (Genbank release 19.0) failed to reveal a bovine sequence with sufficient homology to hybridize to the CPE cDNA probes under the conditions of stringency used.

A comparison of the CPE amino-acid sequence with all proteins in the National Biomedical Research Foundation Protein Sequence Database revealed several proteins with regions of homology to CPE. Using two different computer programs, the proteins with the highest degree of homology with bovine CPE are bovine carboxypeptidase B (CPB) and bovine and rat carboxypeptidase A (CPA). The optimal alignment of CPE with bovine CPA and CPB requires the introduction of 14 gaps in CPA, 13 gaps in CPB and 7 gaps in CPE (Fig. 3). Most of these gaps are small, with a difference of only one or two amino acids between the proteins. Amino acids thought to be essential for catalytic activity in CPA and CPB^{14,15} have been highly conserved in CPE. However, the regions thought to be involved with substrate binding^{14,15} are considerably different for the three enzymes. The amino acids in both CPA and CPB which bind Zn^{2+} (His 69, Glu 72 and His 196 of bovine CPA) and those which participate in the enzymatic reaction (Tyr 198, Tyr 248 and Glu 270 of bovine CPA) are found in CPE (Figs 1, 3). In CPA and CPB, an arginine is thought to polarize the carboxyl group of the amide bond being hydrolysed (Arg 127 of CPA; Arg 124 of CPB). In CPE, a lysine is present (Lys 131) which could perform a similar function. Amino acids in CPA and CPB which are believed to contribute to substrate binding include Arg 142 and Asp 251 of bovine CPB, and Arg 145 and Ile 255 of bovine CPA. The arginine residue is involved in binding to the carboxylate groups of the substrate, and has been conserved in CPE. The other amino acid, which is thought to bind to the substrate side chain, has not been conserved between the enzymes (Figs 1, 3).

Major differences between CPE and the other carboxypeptidases include an additional 120 amino acids at the carboxy-terminus of CPE, and two internal regions of CPE with little homology to CPA or CPB. Both of these internal regions are considerably larger in CPE than in either CPA or CPB. If

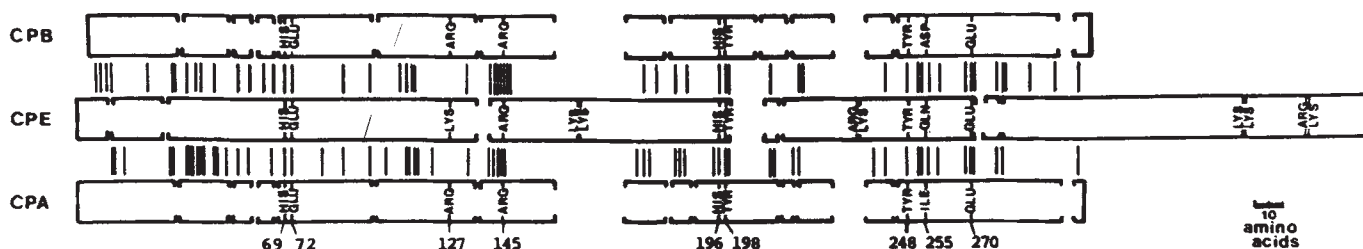


Fig. 3 Schematic representation of homology between bovine carboxypeptidase E, carboxypeptidase B and carboxypeptidase A. Optimum alignment of the amino-acid sequences requires gaps, which are indicated by spaces. Amino acids in carboxypeptidase E which are present in comparable positions within the other enzymes are indicated by a solid line. Amino acids that are important in substrate binding or catalytic activity of carboxypeptidase A and B are shown. The position of these amino acids within CPA is indicated.

CPE has a similar three-dimensional protein structure to CPA and CPB, the two additional internal peptides in CPE would be located near the substrate-binding site. This could contribute to the substantial differences in the substrate specificity of the enzymes; unlike CPA and CPB, CPE does not bind aromatic or aliphatic amino acids¹. Also, CPE is maximally active at pH 5.6 (ref. 1) whereas CPA and CPB have neutral pH optima¹⁶.

The overall homology between CPE and CPA (20%) and between CPE and CPB (17%) is much lower than the homology between CPA and CPB (48%), suggesting that CPE diverged from an ancestral precursor form of CPA and CPB. As the biosynthesis of many neuropeptides requires carboxypeptidase activity, it is likely that this processing enzyme evolved together with the first prohormones. This is supported by the finding that an enzyme very similar to bovine CPE is present in frog, shark and *Aplysia* neural tissues (L.D.F. and E.H., in preparation). Taken together with the evidence that CPE is present in various neurosecretory cells, this observation suggests that CPE is a general enzyme for processing neuropeptides.

Note added in proof: A cDNA clone has now been isolated

which is longer in the 5' direction. The sequence of this clone reveals that CPE must be initially synthesized as a precursor (prepro CPE).

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A novel selective broad-spectrum anti-DNA virus agent

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A new compound has been found, (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine ((S)-HPMPA), that has potent and selective activity against a broad spectrum of DNA viruses, including herpes simplex virus (types 1 and 2); varicella zoster virus; thymidine kinase-deficient (TK⁻) mutants of herpes simplex and varicella zoster virus; human cytomegalovirus; phocid, simian, suid, bovid and equid herpesviruses; African swine fever virus; vaccinia virus; and human adenoviruses. It is also active against retroviruses. We also report that, in mice and rabbits *in vivo*, the compound is effective against both local and systemic infections with herpes simplex virus type 1, including herpetic keratitis caused by a TK⁻ mutant which is resistant to the classical anti-herpes drugs.

With the advent of selective antiviral agents such as acyclovir (ACV) and bromovinyldeoxyuridine (BVDU), antiviral chemotherapy has come of age^{1,2}. Nucleoside analogues such as these are particularly effective against herpes simplex virus (HSV) and varicella zoster virus (VZV) and are selective primarily because of preferential phosphorylation by the virus-encoded thymidine kinase (TK). On conversion to their triphosphate form, ACV and BVDU act as inhibitors of the viral DNA polymerase, and the resulting inhibition of viral DNA synthesis, together with the incorporation of BVDU and ACV into viral DNA (which for ACV can only occur at the 3'-terminal), may account for the antiviral action of ACV and BVDU³.

DNA viruses such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV), which do not encode a specific viral TK, or mutant strains of HSV or VZV which are either deficient in TK-inducing activity (TK⁻) or induce a TK with diminished activity or altered substrate specificity⁴, are relatively insensitive to viral TK phosphorylation-dependent compounds such as

ACV and BVDU. CMV infection is a major cause of death in immunosuppressed patients such as recipients of bone-marrow transplants and those with acquired immune deficiency syndrome (AIDS); EBV causes infectious mononucleosis and is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma and certain lymphomas in immunosuppressed patients; and TK⁻ mutants of HSV or VZV may arise with the widespread use of compounds such as ACV, and, indeed, ACV-resistant (ACV^r) HSV mutants have occasionally been isolated from immunosuppressed patients treated with acyclovir⁵⁻⁸.

Several new acyclic guanosine analogues have recently been synthesized: 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, also termed BOLF-62), BW B759U and NDG (2'-nor-2'-deoxyguanosine)⁹⁻¹²; 9-(3,4-dihydroxybutyl)guanine (DHBG¹³); and 9-(2,3-dihydroxy-1-propoxymethyl)guanine (iNDG¹⁴). DHPG is clearly more effective against human CMV in cell culture¹⁵⁻¹⁷, and clinical studies with DHPG in the treatment of CMV infections in immunosuppressed patients have recently begun¹⁸⁻²⁰.

The novel acyclic adenosine analogue (S)-HPMPA (Fig. 1) is inhibitory to a broad spectrum of DNA viruses, including HSV-1 and -2, TK⁻ HSV-1, VZV, TK⁻ VZV, human CMV, phocid herpesvirus type 1 (seal herpesvirus, SeHV), the simian

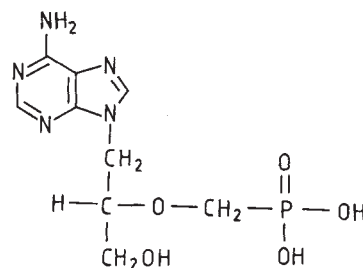


Fig. 1 (S)-HPMPA.

herpesvirus platyrrhinae (HVP), suid herpesvirus type 1 (SHV-1, or pseudorabies virus or Aujeszky's disease virus), bovid herpesvirus type 1 (infectious bovine rhinotracheitis virus, BHV-1), equid herpesvirus type 1 (equine abortion virus, EHV-1), African swine fever (ASF) virus, vaccinia virus, human adenoviruses, and retroviruses such as murine sarcoma virus (MSV) at concentrations far less than those required to affect the metabolism (DNA, RNA or protein synthesis) of the uninfected host cells. (S)-HPMPA is the 2-phosphonylmethyl derivative of (S)-9-

Table 1 Antiviral activity of (S)-HPMPA in cell culture

Virus	Cell culture	MIC ₅₀ (µg ml ⁻¹)			ACV
		(S)-HPMPA	(S)-DHPA	BVDU	
HSV-1 (KOS)	PRK	1	>400	0.02	0.2
HSV-1 (F)	PRK	1-3	>400	0.02	0.2
HSV-1 (McIntyre)	PRK	1-3	>400	0.02	0.1
HSV-2 (G)	PRK	1-3	>400	2	0.07
HSV-2 (196)	PRK	1-3	>400	70	0.07
HSV-2 (Lyons)	PRK	1	>400	7	0.07
TK ⁻ HSV-1 (B2006)	PRK	1	>400	>200	70
TK ⁻ HSV-1 (ACV ^r , BVDU ^r)	E ₆ SM	0.7	>400	>100	70
HSV-1 (PAA ^r -1)	PRK	0.3	>400	0.02	0.2
HSV-1 (PAA ^r -5)	PRK	0.3	>400	0.02	2
VZV (YS)*	HEL	0.004	10	0.006	0.2
VZV (Oka)*	HEL	0.004	70	0.007	0.3
TK ⁻ VZV (YSR)*	HEL	0.004	200	>400	20
TK ⁻ VZV (07-1)*	HEL	0.004	200	>400	4
CMV (Davis)*	HEL	0.3	70	300	20
CMV (AD 169)*	HEL	0.3	200	200	30
SeHV	SeK	1	>300	>300	>300
HVP (MV-5-4)	PRK	2	>400	0.02	10
SHV-1	PRK	2	>400	0.07	7
BHV-1 (LA)	PRK	2	>400	0.2	70
EHV-1 (Kentucky D)	PRK	0.2	>400	>400	7
ASF virus	Vero	0.01	10	—	—
Vaccinia virus	PRK	0.3	50	7	70
Adenovirus (types 1-8)	HEL	0.6-2.5	>125	>125	>125
MSV (Moloney)	MO	1	>200	>100	50

(S)-HPMPA was added 1 h after the cells (in microtray wells) had been infected with either 100 CCID₅₀ (1 CCID₅₀ dose needed to infect 50% of cells) or 20 PFU (plaque-forming units), the latter for those viruses that are marked with an asterisk. Antiviral activity is expressed as MIC₅₀. Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus-infected cell cultures. The standard antiviral compounds (S)-DHPA, BVDU and ACV were included in the assays for comparison. Cell cultures: PRK, primary rabbit kidney; HEL, human embryonic lung; E₆SM, (human) embryonic skin-muscle; MO, C₃H mouse embryo; SeK, seal kidney. Specific virus strains or mutants are in parentheses; for the clinical TK⁻ HSV-1 isolate, the phenotypic designation (ACV^r, BVDU^r) is used. The methodology for growing the virus stocks and for measuring inhibition of virus-induced cytopathogenicity or plaque formation has been described previously^{32,33}; the origin of the HSV-1, HSV-2 and VZV strains, including the TK⁻ VZV mutants, is described in refs 32 and 33. The human CMV strains were provided by Dr S. Chiba. The HVP, MV-5-4 (ATCC VR-349), SHV-1 (ATCC VR-135), BHV-1 (LA (ATCC VR-188)) and EHV-1 (Kentucky D ATCC VR-700) strains were obtained from the American Type Culture Collection (Rockville). The PAA^r-1 and PAA^r-5 mutants of HSV-1 were provided by Dr C. Knopf; these mutants were originally described by Chartrand *et al.*³⁴ and Coen *et al.*³⁵, respectively. Their mutations map within the HSV DNA polymerase locus and confer resistance to phosphonacetic acid (PAA) and phosphonoformate (PFA). The TK⁻ HSV-1 B2006 mutant was provided by Dr Y.-C. Cheng; it was originally described by Dubbs and Kit³⁶. All HSV-1 and VZV strains indicated as TK⁻ are truly TK-deficient, as they do not induce TK activity above the base levels of TK activity found in mock-infected PRK or HEL cells. The TK⁻ HSV-1 (ACV^r, BVDU^r) variant was isolated from an immunosuppressed patient who had been treated with ACV for an orofacial HSV-1 infection and whose clinical condition deteriorated despite antiviral treatment. All HSV-1 isolates obtained from this patient following ACV treatment were resistant to ACV, BVDU and several other anti-herpes drugs, such as 5-ethyl-2'-deoxyuridine and CEDU^{37,38}. In contrast, the pre-therapy HSV-1 isolate showed a similar sensitivity to the anti-herpes drugs as wild-type HSV-1 strains HSV-1 (KOS). The HSV-1 (ACV^r, BVDU^r) variant was designated⁴ as TK⁻, as it induces only 1-3% of the TK activity induced by the pre-therapy HSV-1 isolate or the wild-type HSV-1 (KOS), in TK⁻ HeLa cells (data not shown).

(2,3-dihydroxypropyl)adenine ((S)-DHPA)²¹, from which it is prepared by an etherification reaction. In contrast with (S)-DHPA, which is primarily active against (-)RNA strand viruses (rabies, vesicular stomatitis, parainfluenza and measles virus), and double-stranded RNA viruses (such as reo- and rotavirus)²², (S)-HPMPA is not active against RNA viruses other than retroviruses.

We explored the antiviral activity of (S)-HPMPA *in vitro* in several cell culture systems and with several viruses (Table 1). (S)-HPMPA inhibits TK⁺ and TK⁻ HSV strains, and TK⁺ and TK⁻ VZV strains, to approximately the same extent. This contrasts markedly with (S)-DHPA (which is not inhibitory to most of the viruses tested) and BVDU and ACV (significantly less inhibitory to the TK⁻ mutants than to the TK⁺ HSV or VZV strains). At 0.3 µg ml⁻¹, (S)-HPMPA is active against the DNA polymerase-based HSV-1 mutants PAA^r-1 and PAA^r-5, whereas neither phosphonacetic acid (PAA) nor phosphonoformate (PFA) are inhibitory to these mutants at concentrations up to 200 µg ml⁻¹. (S)-HPMPA is also effective against several herpesviruses of veterinary importance such as SHV-1, BHV-1 and EHV-1; BVDU is entirely inactive against the latter (Table 1). We also evaluated (S)-HPMPA for its activity against the RNA viruses vesicular stomatitis, parainfluenza type 3, measles, reo type 1, Sindbis, Semliki Forest, Coxsackie type B4 and polio type 1, but found that none of these is sensitive to (S)-HPMPA. (S)-HPMPA is inhibitory, however, to the transformation of mouse embryo cells by MSV, and so is the closely related 9-(2-phosphonylmethoxyethyl)adenine (PMEA). This means

that (S)-HPMPA, PMEA and acyclic adenylate derivatives in general are potential anti-retrovirus agents and should be explored further for their inhibitory effects on the AIDS virus.

(S)-HPMPA is active at 0.3 µg ml⁻¹ against SeHV, a highly pathogenic herpesvirus from the harbour seal²³, which is not inhibited by (S)-DHPA, BVDU and ACV at concentrations up to 100 µg ml⁻¹ (Table 1; A.D.M.E. Osterhaus, J. Groen and E. De C., manuscript in preparation). (S)-HPMPA is active at 0.6-2.5 µg ml⁻¹ against human adenovirus types 1-8; neither (S)-DHPA nor BVDU and ACV are active against human adenovirus at concentrations up to 125 µg ml⁻¹ (Table 1; M. Baba, S. Mori, S. Shigeta and E. De C., manuscript in preparation). (S)-HPMA is by far the most active inhibitor of the iridovirus, African swine fever virus (MIC₅₀ = 0.01 µg ml⁻¹, where MIC₅₀ is the minimum inhibitory concentration giving 50% inhibition) among many broad-spectrum antiviral agents tested, including (S)-DHPA (Table 1; C. Gil-Fernandez and E. De C., unpublished data). The effect of (S)-HPMPA on EBV replication is currently under investigation by J. C. Lin and J. S. Pagano.

Although inhibitory to HSV, VZV, CMV, SeHV, HVP, SHV, BHV, EHV, MSV, ASF virus, vaccinia virus and adenoviruses at a concentration of ≤1-2 µg ml⁻¹, (S)-HPMPA does not affect normal host-cell metabolism at concentrations ≥100 µg ml⁻¹. Thus, the MIC₅₀ of (S)-HPMPA for DNA synthesis (monitored by the incorporation of 2'-[Me-³H]deoxythymidine), or RNA synthesis (monitored by the incorporation of [5-³H]uridine), or

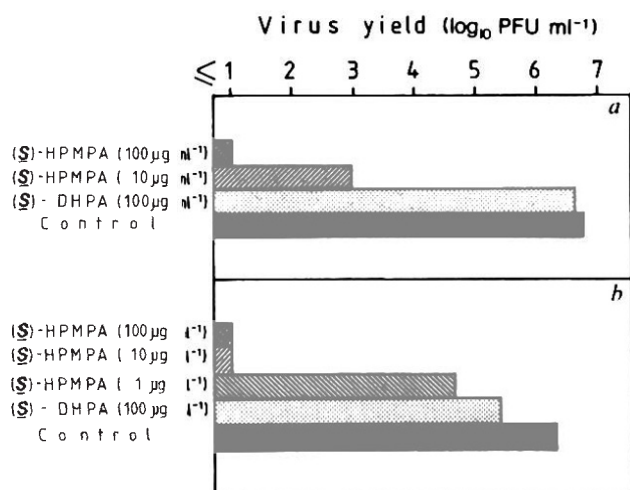


Fig. 2 Effect of (S)-HPMPA and (S)-DHPA on multiplication of HSV-1 (KOS) and vaccinia virus in primary rabbit kidney cells. Cells were infected with HSV-1 (KOS) (a) or vaccinia virus (b) ($4.5 \log_{10}$ PFU per 0.5 ml per Petri dish) and, after 1 h adsorption of the virus, incubated in the presence of the test compounds. Virus yield was measured at 24, 48 and 72 h after infection. Cell cultures were frozen at -70°C , thawed, and the cell homogenates assayed for virus content by plaque formation (PFU) in Vero (green monkey kidney) cells. Only the virus yields measured at 24 h are shown, but similar results were obtained with the yields measured at 48 or 72 h after infection.

protein synthesis (monitored by the incorporation of $[4,5\text{-}^3\text{H}]\text{-leucine}$) in uninfected PRK cells is 100, >200 and $>200 \mu\text{g ml}^{-1}$, respectively.

That the inhibitory effects of (S)-HPMPA on virus-induced cytopathogenicity (Table 1) reflect inhibition of virus multiplication was ascertained by measuring virus growth in PRK cell cultures that had been inoculated with vaccinia virus or HSV-1 (KOS) and subsequently exposed to (S)-HPMPA (Fig. 2). At $100 \mu\text{g ml}^{-1}$, (S)-HPMPA completely suppresses the growth of HSV-1 (KOS) and vaccinia virus. At $10 \mu\text{g ml}^{-1}$, it completely suppresses the growth of vaccinia virus and reduces the yield of HSV-1 (KOS) by almost $4 \log_{10}$ units. Even at $1 \mu\text{g ml}^{-1}$, (S)-HPMPA causes a more significant reduction in vaccinia virus yield than does (S)-DHPA at $100 \mu\text{g ml}^{-1}$ (Fig. 2).

We assessed the antiviral activity of (S)-HPMPA *in vivo* using: (1) a cutaneous HSV-1 model infection in hairless mice; (2) a lethal systemic HSV-1 infection in mice; and (3) a herpetic eye infection model in rabbits. (S)-HPMPA is efficacious in all three models. In hairless mice inoculated intracutaneously (i.c.) with HSV-1 (KOS), (S)-HPMPA completely suppresses the development of herpetic skin lesions when applied topically (4 times a day) from day 0 until day 4 post-infection at 0.1, 0.3 or 1% in dimethyl sulphoxide (DMSO) and thereby reduces the mortality rate from 100% to 10, 0 and 0%, respectively (Table 2). In mice infected intraperitoneally (i.p.) with HSV-1 (KOS), (S)-HPMPA reduces the mortality rate from 100% to 60 or 30% when administered perorally (via gavage, twice a day, from day 0 to day 4 post-infection) at a dosage of 50 or 250 mg per kg per day, respectively (Table 2). In rabbits inoculated corneally with the TK⁻ HSV-1 (ACV^r, BVDU^r) strain, (S)-HPMPA eyedrops at 0.2% completely heal the corneal lesions under conditions where 0.2% BVDU eyedrops and 0.2% 5-(2-chloroethyl)-2'-deoxyuridine (CEDU) eyedrops have no effects on the progression of the disease. In the treatment of herpetic keratitis caused by the TK⁺ HSV-1 McIntyre strain, 0.2% (S)-HPMPA eyedrops are equally effective as 0.2% BVDU eyedrops and 0.2% CEDU eyedrops (P.C.M., E. De C. and P. Huyghe, in preparation).

Table 2 Antiviral activity of (S)-HPMPA in mice

Compound	No. of surviving mice following virus infection (days)					
%	0	5	7	9	15	20
Model a						
(S)-HPMPA 1	10	10 (0, 0)	10 (0, 0)	10 (0, 0)	10 (0, 0)	10 (0, 0)
(S)-HPMPA 0.3	10	10 (0, 0)	10 (0, 0)	10 (0, 0)	10 (0, 0)	10 (0, 0)
(S)-HPMPA 0.1	10	10 (0, 0)	10 (0, 0)	10 (0, 0)	9 (0, 0)	9 (0, 0)
(RS)-DHPA 10	10	9 (4, 1)	4 (2, 2)	1 (1, 0)	1 (1, 0)	1 (1, 0)
Placebo	10	1 (6,3)	1 (1, 0)	1 (0, 1)	0	0
Model b						
(mg per kg per day)						
(S) HPMPA 250	10	10	10	10	7	7
(S)-HPMPA 50	10	10	8	5	4	4
(S)-HPMPA 10	10	9	6	1	1	1
(RS)-DHPA 250	10	10	4	2	2	2
Placebo	10	10	3	0	0	0

For model a, 25–30-day-old hairless (*hr/hr*) mice (15–20 g) were inoculated i.c. in the lumbosacral area with HSV-1 (KOS) at $10^{4.7}$ PFU per 0.05 ml per mouse and treated topically at the indicated concentrations of the compounds (formulated in DMSO), 4 times a day for 5 days, starting immediately after virus infection. Numbers in parentheses refer to the number of mice with skin lesions, followed by number of mice with skin lesions plus paralysis of the hind legs. For model b, 25-day-old NMRI (Naval Medical Research Institute) mice (11–13 g) were inoculated i.p. with HSV-1 (KOS) at 10^3 PFU per 0.2 ml per mouse and treated perorally (by gavage) at the indicated doses of the compounds (dissolved in drinking water), twice a day for 5 days, starting on the day of virus infection.

In addition to (S)-HPMPA, other acyclic adenosine phosphonylmethyl derivatives were synthesized and examined for antiviral potential. (The detailed analysis of the structure-function relationship of this class of compounds will be reported elsewhere.) The PMEA mentioned above is about as active as (S)-HPMPA against TK⁺ HSV and TK⁻ HSV but significantly less active against VZV, CMV and vaccinia virus.

The 3'-O-phosphonylmethyl derivative of (S)-DHPA is markedly less active than (S)-HPMPA, whereas the 2'- and 3'-O-phosphonylmethyl derivatives of (R)-DHPA are virtually inactive. The mixture of the 2'- and 3'-O-phosphonylmethyl isomers derived from racemic DHPA, as well as the mixture of 2'- and 3'-isomers derived from (S)-DHPA, are also active, although to a lesser extent than pure (S)-HPMPA.

Phosphonylalkyl derivatives of heterocyclic bases have recently been synthesized and shown to inhibit purine nucleoside phosphorylase²⁴. Tetrazole phosphonic acids (5-(phosphonomethyl)-1H-tetrazole) are very weak antiviral agents²⁵, and phosphonylated derivatives of ACV have been accredited with anti-HSV activity²⁶. These compounds differ fundamentally from (S)-HPMPA and PMEA in that they lack the ether linkage between the alkyl residue and the phosphorus atom.

The metabolic fate and mechanism of action of (S)-HPMPA remain unknown. The ether linkage means that the compound cannot be metabolized to PAA or PFA. If the antiviral activity of (S)-HPMPA is caused by the formation of PFA or PAA, (S)-HPMPA would be expected to be inactive against the PAA^r-mutants of HSV-1, but this does not prove to be the case. Note that only the (S)-enantiomer of HPMPA is antivirally active, whereas the (R)-enantiomer is not (or markedly less) active. This means that the mechanism of antiviral activity of HPMPA is based on its chirality.

As the antiviral activity spectrum of (S)-HPMPA is totally different from that of (S)-DHPA and other adenosine analogues that are targeted at S-adenosylhomocysteine hydrolase^{22,27,28}, the latter enzyme can be excluded as the target for the antiviral action of (S)-HPMPA. In fact, *in vitro* assays with purified rat liver S-adenosylhomocysteine hydrolase prove that (S)-

HPMPA does not inhibit the enzyme (our data, not shown). From the data presented here, it is obvious that the antiviral activity of (S)-HPMPA is not dependent on the help of a virus-specified TK. In this sense, (S)-HPMPA resembles 2'-nor-cyclic GMP, the cyclic phosphate of NDG, which is also endowed with broad-spectrum anti-DNA virus activity²⁹. As (S)-HPMPA cannot be enzymatically dephosphorylated, (S)-HPMPA may be taken up by the cells in this form and phosphorylated intracellularly by cellular kinases, as has been demonstrated recently for DHPG homophosphonate^{30,31}. The active form of (S)-HPMPA could be its diphosphoryl derivative, and in this form it may be targeted at the viral DNA polymerase. In its native form (S)-HPMPA would not then interact with the viral DNA polymerase, as suggested by its inability to block at concentrations up to 400 $\mu\text{g ml}^{-1}$ the reverse transcriptase activity associated with Moloney murine leukaemia virus (our data, not shown).

(S)-HPMPA has a potent and selective inhibitory activity against several viruses which are normally insensitive or may have become insensitive to currently available antiviral drugs. Its broad-spectrum anti-DNA virus activity, combined with its efficacy in animal model systems and apparent lack of toxicity at the active dosage schedules, make it a promising antiviral agent for the treatment of various DNA virus and retroviral infections.

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Suppression of mouse viraemia and retroviral disease by 3'-azido-3'-deoxythymidine

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The retroviruses human T-cell lymphotropic virus-I (HTLV-I)^{1,2} and HTLV-III/LAV (lymphadenopathy-associated virus)^{3,4} are clearly linked to human diseases. Patients with HTLV-I-positive neoplasms may respond transiently to traditional chemotherapy, but are not cured⁵. For patients with acquired immune deficiency syndrome (AIDS) there is no curative therapy⁶. In retroviruses of different species, viral propagation crucially depends on reverse transcriptase, an enzyme not present in normal mammalian cells and different from mammalian DNA polymerases, making it a target for specific inhibition. Reverse transcriptase has been well conserved through evolution: an LAV isolate contained a 250-amino-acid-long domain, presumably the reverse transcriptase core sequence, which has 21% homology to Moloney murine leukaemia virus (MoMLV)⁷. Because HTLV-III infects only humans and chimpanzees⁸, we substituted murine retroviruses for *in vivo* evaluation of candidate anti-AIDS drugs after ascertaining similar inhibition *in vitro* of HTLV-III and MLVs, which were chosen for their short incubation time. The triphosphate of 3'-azido-3'-deoxythymidine (AZT)⁹⁻¹¹ is incorporated into complementary DNA by retroviral reverse transcriptase, causing premature chain termination. Here we show that chronic AZT treatment of mice infected with Rauscher murine leukaemia virus complex (RLV) prevents infection of splenocytes and development of splenomegaly, and suppresses viraemia if started soon after inoculation. Starting AZT late in the course of disease still leads to significant prolongation of life; anaemia, however is a significant side-effect. By analogy, AZT may have a role in preventing retroviral disease in humans if started early after infection, and it may lead to significant survival gains even if started later in the course of disease.

AZT, after intracellular phosphorylation to AZT triphosphate, competitively inhibits reverse transcriptase with regard to thymidine triphosphate¹¹, whereas mammalian DNA polymerases do not readily accept AZT triphosphate as substrate. AZT effectively inhibits infectivity and cytopathic effects of HTLV-III *in vitro* with little cytotoxicity¹¹, and improves immunological function in AIDS patients given short-term courses of AZT¹².

We sought to test whether prompt initiation of AZT therapy suppresses viraemia, leading to survival benefits for BALB/c mice infected with RLV^{13,14}. This virus attacks red cell precursors, causes abnormal splenic colony formation in 8 days, massive splenomegaly in 3 weeks and kills infected animals in 4-5 weeks. Numbers of spleen colonies on day 8 and spleen weight on day 20 are functions of viral titre¹⁵. With this model, candidate drugs can be evaluated rapidly for therapeutic effectiveness against retrovirus-induced disease and toxicity¹⁶.

In the XC plaque assay¹⁷, AZT inhibited plaque formation by RLV with an inhibitory concentration₅₀ (IC₅₀) of 6.6 nM and that by the T-cell tropic SL3-3 MLV¹⁸ with an IC₅₀ of 12.2 nM, without appreciable cytotoxicity. All AZT regimens led to significant inhibition of splenomegaly (Table 1). To test whether AZT could render spleen cells of treated infected mice non-infectious, we analysed single-cell suspensions by XC infectious centre assay^{16,19}. The percentage of infectious splenocytes decreased from 96 for infected control mice by ~3 logarithm units for mice given the lower intraperitoneal (i.p.) doses. No infectious cells were detected at 80 mg kg⁻¹ i.p. or with orally

Table 1 Effects of AZT treatment on spleen weights and viral titres

Cage No.	No. of Mice	Virus	Treatment with AZT	Mean spleen weight (mg)	% Inhibition splenomegaly*	% infectious cells	PFU ml ⁻¹ plasma	AZT level (μM) at death
1, 2	14	+	none	1,786 ± 646		96	4.2 × 10 ⁵	
3	6	-	none	120 ± 17		0		
4	7	-	20 mg kg ⁻¹	72 ± 14				
5	8	+	i.p. Q 8 h	177 ± 75	94	0.005	1.4 × 10 ³	1.53
6	8	-	40 mg kg ⁻¹	82 ± 26				
7	8	+	i.p. Q 8 h	121 ± 33	98	0.13	2.3 × 10 ³	0.6
8	8	-	80 mg kg ⁻¹	54 ± 6				
9	8	+	i.p. Q 8 h	99 ± 30	97	<0.001	<100	0.67
10	8	-	0.5 mg ml ⁻¹	100 ± 17				
11	8	+	in drinking water	199 ± 32	94	<0.001	<100	1.65
12	8	-	1.0 mg ml ⁻¹	86 ± 7		<0.001		
13	8	+	in drinking water	127 ± 25	98	<0.001	<100	8.94

Female BALB/c mice (6 weeks old) were treated according to the above schedules. Saline or 1×10^4 plaque-forming units (PFU) of RLV, strain RVB3 were given on day 0, followed 4 h later by AZT i.p. every 8 h or in the drinking water at the doses indicated. Water was withheld from the mice for 8 h before AZT treatment. The dose 1 mg ml^{-1} AZT in drinking water corresponds to about $145 \text{ mg kg}^{-1} \text{ day}^{-1}$. On day 20 post-inoculation, the mice were killed after obtaining weight and blood samples, and mean spleen weights were determined. Per cent inhibition of splenomegaly was calculated by using the formula $[1 - (x - y)/(c - n)] \times 100$. x , Mean spleen weight of mice given virus plus AZT; y , that of mice given AZT at the same dose only; c , that of virus-infected control mice; and n , that of normal untreated mice.

* Using a two-tailed t test, comparison of mean spleen weights for cages 5, 7, 9, 11 or 13 with that of virus-infected mice yield $P < 0.0001$ for each group. The percentage of infectious cells was determined from single-cell suspensions prepared from aseptically removed spleens in Dulbecco's modified Eagle's medium containing 20% fetal calf serum. After dilution, 10^2 – 10^5 nucleated cells were plated onto SC-1 cells for the infectious centre assay¹⁹. No plaques were seen for groups 8, 9, 11, 12 and 13. Viral titres were analysed after a 1:10 dilution of plasma in phosphate-buffered saline, passage through a 0.45 Nalgene filter and storage in liquid nitrogen. The plasma was subsequently tested at dilutions ranging from 10^{-2} – 10^{-5} in the XC plaque assay¹⁷. The higher dilutions were sufficient to lower the AZT contained in the plasma originally to levels below the IC_{50} obtained *in vitro*. No plaques were seen for groups 9, 11 and 13. Blood samples for AZT levels for cages 5, 7 and 9 were collected 3–4 h after the last dose and 1 h after removing the AZT-containing water from cages 11 and 13. Serum AZT levels were analysed according to the method of S. Good *et al.* (personal communication). AZT was provided by the Burroughs Wellcome Company.

administered AZT. Plasma viral titres yielded similar results, which were not caused by residual AZT in test samples: although a 100-fold dilution of plasma from mice given 80 mg kg^{-1} AZT i.p. equalled the IC_{50} of 6.6 nM obtained *in vitro*, no plaques were seen at dilutions between $1:10^2$ and $1:2 \times 10^5$. To confirm these results, 1 mg ml^{-1} AZT was given to RLV-infected mice in drinking water and treatment was stopped on day 20 post-inoculation. No virus was detected 1, 9 and 49 days after stopping AZT. Thus, chronic oral AZT treatment beginning 4 h after infection suppressed viraemia. The short half-life of AZT in mice (30–60 min; S. Good, unpublished observation) led to clinically subinhibitory levels when AZT is given i.p. every 8 h at the lower two doses.

AZT toxicity was tolerable during the 20 days of therapy. Clinically, all treated mice remained well. Analysis revealed moderate anaemia and depression of white cell counts (Table 2). Elevation of alkaline phosphatase was also seen, but bilirubin and serum creatinine remained normal (not shown).

The effect of long-term AZT treatment on survival of RLV-infected mice was analysed at 1 mg ml^{-1} in drinking water, corresponding to about $145 \text{ mg kg}^{-1} \text{ day}^{-1}$. Infected control animals had a median survival of 26 days. One infected, AZT-treated mouse died of toxicity after 53 days. After 55 days, all animals started on AZT 4 h post-inoculation were killed because of drug toxicity (Table 2). Three of 4 mock-infected mice receiving AZT develop >20% weight loss; severe depression of white and red cells; and corneal opacities. Nonetheless, the survival advantage of AZT-treated compared with control infected mice is significant ($P = 0.03$, Fig. 1a).

Treatment with a lower dose of AZT (0.1 mg ml^{-1} in drinking water, equivalent to $15 \text{ mg kg}^{-1} \text{ day}^{-1}$) started 4 h post-inoculation led to significant prolongation of life: no deaths occurred within 24 weeks in infected, AZT-treated mice, whereas control animals given the same inoculum have a median survival of 3.6 weeks ($P < 0.0001$, Fig. 1b). Bone marrow depression did not occur, but the lower dose of AZT did not prevent splenomegaly. Spleen

Table 2 Haematological parameters

Cage No.	Dose AZT	Day collected	WBC × 10 ³	Hb (g dl ⁻¹)	Hct (%)	Plts (×10 ⁶)	Total bili (mg dl ⁻¹)
1, 2	none	21	32.3	9.7	29.5	417	ND
3	none	20	6.8	15.8	48.2	1,040	0.5
		55	7.5	16.0	48.0	1,124	ND
4	20 mg kg ⁻¹ i.p. Q 8 h	20	11.0	14.6	42.9	1,262	0.4
9	80 mg kg ⁻¹ i.p. Q 8 h	20	3.6	10.9	31.5	1,792	0.5
10	0.5 mg ml ⁻¹ in drinking water	20	8.6	13.6	39.6	1,114	0.5
11	0.5 mg ml ⁻¹ in drinking water	20	5.4	13.3	39.8	1,428	0.5
12	1.0 mg ml ⁻¹ in drinking water	20	7.8	11.5	32.2	1,367	0.6
		55	2.8	1.7	4.9	1,671	ND
13	1.0 mg ml ⁻¹ in drinking water	20	4.5	10.3	28.7	1,400	0.5
14	0.1 mg ml ⁻¹ in drinking water	46	8.6	15.6	46.7	1,469	ND
		65	7.1	15.8	46.7	1,165	ND
		156	9.5	15.9	45.1	1,506	ND

Blood was obtained by collecting samples from the tails of mice on the day post-inoculation indicated. Blood from several mice was pooled. WBC, white blood count per mm³; Hb, haemoglobin; Hct, haematocrit; Plts, platelet count per mm³. Values for cages 1, 2 and 14 were obtained from a parallel experiment. ND, not done.

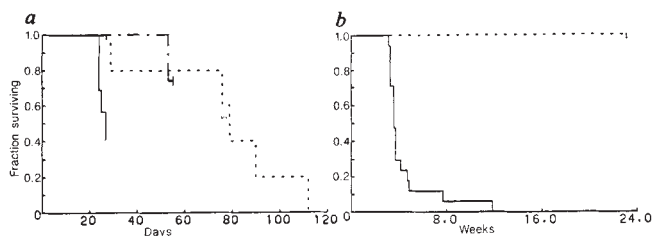


Fig. 1 Survival of RLV-infected mice with or without AZT treatment. *a*, Kaplan-Meier plots of virus-infected, untreated mice (—), or infected mice treated with 1 mg ml⁻¹ AZT in drinking water starting 4 h (---) or 19 days (····) post-inoculation, sample sizes 16, 8 and 10 mice respectively. Approximate AZT doses are 145 (---) and 64.5 mg kg⁻¹ day⁻¹ (····) as infected mice consumed about half as much fluid as uninfected controls because of the splenomegaly that had developed by day 19. *b*, As *a*, but AZT treatment given at 0.1 mg ml⁻¹ in drinking water corresponding to 15 mg kg⁻¹ day⁻¹. All treatment groups contained 16-17 mice. Controls, untreated mice (no deaths in *a* or *b*) or uninfected mice given AZT at appropriate doses. No deaths occurred for AZT at 0.1 mg ml⁻¹; for 1 mg ml⁻¹ drug deaths did occur (see text). *P* values (calculated by log-rank test): 0.03 for AZT at 1 mg ml⁻¹ started either at 4 h or 19 days (*a*); and <0.0001 for AZT at 0.1 mg ml⁻¹ started at 4 h (*b*).

weights measured on day 21 post-inoculation ranged from 151 to 586 mg (mean 275 mg) in AZT-treated, infected mice, 89 ± 13 mg in AZT-treated, uninfected mice and 1,836 ± 309 mg in infected untreated mice.

AZT suppression of splenomegaly, infection of spleen cells and viraemia can be explained by several mechanisms, the most likely being inhibition of DNA synthesis by reverse transcriptase via premature chain termination. Alternatively, AZT could interfere with release of mature virions from productively infected cells. Release of radioactively labelled SL3-3 virus from murine T-cell cultures grown in the presence or absence of AZT was measured. At the highest AZT concentration there was only a 22% decrease (Fig. 2) which did not change after growing virus-producing cells for 30 days in AZT. Thus, AZT had only minor influence on steps leading from transcription of proviral sequences to release of viral particles.

AZT could also be preferentially toxic to virally infected, transformed cells. Because we observed moderate anaemia, AZT may be cytotoxic to RLV target cells (red cell precursors). To test antineoplastic action of AZT, RLV-infected mice were allowed to develop massive splenomegaly. On day 19 post-inoculation, ~1 week before death for 50% of infected mice, treatment with AZT at 1 mg ml⁻¹ (Fig. 1*a*) began and was continued until death. After discontinuing AZT (2 h) virus could be recovered from infected mice treated between days 19 and 27 with AZT at this dose. Although all mice eventually died of disease with massive splenomegaly, there was a significant survival advantage (Fig. 1*a*, median survival, 77.5 days, *P* = 0.03). Thus, although AZT did not stop viraemia nor prevent eventual death from leukaemia, it significantly stabilized the disease.

We have demonstrated AZT to be an active antiretroviral agent capable of suppressing infection and disease in mice treated 4 h after RLV infection, but therapeutic doses lead to severe haematological depression after prolonged therapy. As reverse transcriptase inhibitors generally act as virustatic rather than virucidal agents¹⁶, we are now testing whether interruption of therapy to prevent toxicity will lead to late recurrence of retroviral illness caused by uninhibited amplification of viraemia undetectable in the presence of AZT.

The significant survival advantage of mice chronically treated with oral AZT suggests this drug may be effective in humans infected with pathogenic retroviruses. Thus, we recommend AZT for clinical trials in AIDS patients and for evaluation in patients with HTLV-I-positive neoplasms. However, unlike MLV-induced leukaemias, only a few HTLV-I induced neoplasms express viral RNA sequences²⁰, and infection of new target cells

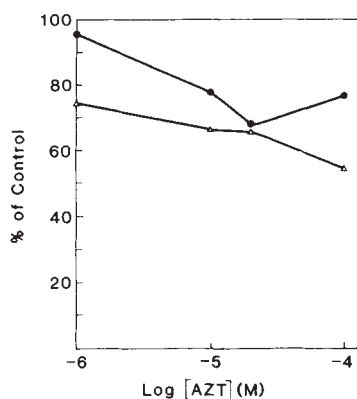


Fig. 2 Release of labelled SL3-3 virus per live T cell cultured in the presence of AZT. Murine L691-6 cells producing high-titre SL3-3 virus were incubated for 36 h with AZT. For the last 12 h, tritiated uridine was added at 10 µCi ml⁻¹. The cells were then counted in Trypan blue, spun out and supernatants clarified at 10,000 g for 15 min. The virus was pelleted in a SW27 rotor at 85,000 g for 90 min and resuspended in TEN (0.05 M Tris-HCl, pH 7.5, 0.001 M EDTA, 0.15 M NaCl) and layered on top of preformed 10-50% linear sucrose gradients in TEN. After centrifugation at 85,000 g for 4.5 h at 4°C, fractions were collected and the refractive indices determined. After adding 80 µg carrier DNA, each fraction was tricarboxylic acid-precipitated onto a nitrocellulose filter and counted. The radioactivity banding at densities between 1.14 and 1.18 gm cm⁻³ was determined for each gradient and correlated to the number of live cells at the end of the 36-h incubation in AZT. Results plotted as per cent of c.p.m. per cell released from cells not exposed to AZT (black circles). Per cent surviving cells shown by triangles.

may be relatively rare. Nevertheless, the poor prognosis of HTLV-1-induced neoplasms may warrant trials of antiviral agents in combination with cytotoxic therapy. Alternatively, AZT could be useful in preventive treatment of health-care workers after accidental exposure to high doses of human retroviruses. An important question raised by our experiments is that of antineoplastic action of AZT against leukaemic cells. We demonstrate that AZT therapy can lead to increased survival in an animal model of retrovirally induced leukaemia, suggesting that AZT should be studied against other types of leukaemias.

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Core formation and Earth's late accretionary history

A RECENT paper by Morgan¹ concerning the origin of refractory noble siderophile elements in the Earth's upper mantle contains statements which may be misleading to interested readers who have not followed in detail the recent debates on core formation and mantle siderophile elements. Morgan used the approximately chondritic Os/Re ratio inferred for the Earth's Mantle from the Os isotopic systematics of terrestrial osmiridites to examine three hypotheses of core formation: (1) heterogeneous accretion/'chondritic veneer'²; (2) equilibrium between mantle silicates and a eutectic Fe-S-O liquid³; and (3) inefficient core formation⁴. Morgan concludes on the basis of both terrestrial and meteoritic Os/Re ratios that the heterogeneous accretion/chondritic veneer (or 'late influx') hypothesis is most likely and should be favoured.

We feel that two points of Morgan's paper deserve further discussion. First, as regards our own model of inefficient core formation, even very small amounts of trapped metal will tend to buffer the residual mantle at the Os/Re ratio which existed before core formation. Secondly, as a consequence of the first point, it is unclear to us whether or not the Os/Re ratio of the present mantle has been modified, relative to the bulk Earth value, by mantle processes such as core formation or extraction of basaltic magma. In the absence of knowledge of the bulk Earth Os/Re ratio, it is not obvious how one establishes that the present Os/Re ratio of the upper mantle reflects the addition of a chondritic 'late-stage veneer', rather than resulting from *in situ* processes.

We disagree with Morgan's evaluation of hypothesis (3). The hypothesis postulates that the approximately chondritic ratios of the refractory noble siderophile elements in the mantle result from the retention of very small amounts of solid metal and S-bearing metallic liquid during segregation of the core from the mantle (that is, core formation was not 100% efficient⁴), with subsequent oxidation of that metal. Morgan¹ states that chondritic ratios of refractory noble siderophile elements are not a natural consequence of this hypothesis. This statement is correct as a generality because fractionations between noble siderophile elements are, in principle, allowed during geologic processes. However, in practice, Morgan's statement is not correct as applied to our model of inefficient core formation because trace amounts of trapped solid metal will tend to buffer the mantle towards the noble metal abundance ratios which existed before core formation. The (solid metal/silicate liquid) and (liquid metal/silicate liquid) partition coefficients

for Os (by analogy with Ir) are $\sim 10^6$ and $\sim 10^4$, respectively, while those for Re are $\geq 10^5$ and $\geq 10^3$, respectively⁵. Thus, if core formation is not completely efficient, trace amounts of trapped solid metal will tend to dominate the other Os-Re reservoirs in the mantle, closely preserving original noble metal abundance ratios in the subsequently oxidized upper mantle.

For example, model calculations indicate that the fractionation of the mantle Os/Re ratio relative to the bulk Earth value during inefficient core formation is less than the range of Os/Re ratios observed in chondritic meteorites. Because Os behaves similarly to Ir in geochemical processes such as core formation⁶ and peridotite partial melting⁷, Os may be preferentially incorporated into the Earth's core relative to Re, resulting in a mantle with a slightly lower Os/Re ratio than the bulk Earth. However, even if the (solid metal/liquid metal), (liquid metal/liquid silicate) and (solid silicate/liquid silicate) partition coefficients for Os are larger than those for Re by factors of 2, 10 and 50, respectively, the Os/Re ratio in the mantle will typically deviate by less than 30% from the original Os/Re ratio—less than the range of the means of the chondrite groups reported by Morgan¹.

This calculation is most sensitive to the relative values of the (solid metal/liquid metal) partition coefficients chosen for Os and Re. The relative constancy of Os/Re and Os/Ir ratios in magmatic iron meteorite groups, whose Os, Re and Ir concentrations change by up to four orders of magnitude⁶, argues that Os, Re and Ir behave rather coherently during core formation and solidification processes. This observation suggests that the factor of two difference which we have used in our sample calculation represents a firm limit on the allowable difference between Os and Re. Of course, if the partition coefficients for Os and Re were identical, no fractionation would occur in metallic systems and the Os/Re ratio of the mantle would remain essentially unchanged during inefficient core formation.

As shown in Morgan's¹ Fig. 1, the Os/Re weight ratio in individual samples of 12 types of chondritic material varies from ~ 8 to ~ 19 (a factor of 2.4), although average values for chondrite groups (which are probably less sensitive to sampling) exhibit a smaller range, from ~ 8 to 14. Evidently there is no exact value of the 'chondritic' Os/Re ratio and, in the absence of convincing independent evidence that the Earth is made of a particular type of chondrite material, it is not possible to determine that the mantle Os/Re ratio has been unaffected by metal/silicate segregation. For example, if the initial bulk Earth Os/Re ratio were identical to that of CI chondrites (Os/Re = 14; ref. 8), a 15% reduction

could change the mantle Os/Re ratio to 12—a value consistent with isotopic and chemical data obtained from mantle-derived materials^{1,9}, and consistent with our upper limit on the likely change in the mantle Os/Re ratio during core formation. (We note that, in an earlier version of this comment, we took the Os/Re ratio of CI chondrites to be 19—a mistake caused by an error in the abundance of Os in Orgueil given in Table 1 of ref. 8. The interested reader should change this figure from 699 to 504 p.p.b. in ref. 8 (E. Anders, personal communication). We thank J. W. Morgan for catching this error of calculation and pointing it out to us.) Thus, because both the exact value of the Earth's Os/Re ratio and the exact amount of fractionation of Os from Re during core formation are unknown, it does not seem possible to regard the mantle Os/Re ratio as pristine simply because the mantle value falls within the chondritic range.

We conclude that Os/Re fractionation between mantle and core is only significant when core formation is efficient and no solid metal (≤ 0.04 wt %) is retained by the mantle. Although minor Os/Re fractionations are possible, their magnitudes are typically less than the range of the mean Os/Re ratios of individual chondrite groups. Even though the Earth's mantle has an Os/Re ratio within the range observed in chondritic materials, that value could still have been altered by core-forming processes and is not necessarily primordial. Ratios of moderately siderophile elements such as Co/Ni do pose problems for inefficient core formation models⁵, but ratios of refractory noble siderophile elements such as Os/Re do not. Of all the evidence for a late-stage chondritic veneer, the Os/Re ratio of the Earth's mantle is, in our view, the least conclusive.

We would like to thank John Morgan for a scholarly, friendly exchange of views, and Ed Anders for clarifying cosmic Os abundances. This work was supported by NASA grant NAG 9-39.

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MORGAN REPLIES—My letter¹ raises two major points. First, the chondritic Os/Re

ratio for undepleted mantle inferred from the Os isotopic evidence fulfils a prediction of the late influx model (LIM) which is not inherent in the inefficient core formation model (ICFM). Second, if the LIM is accepted as a working hypothesis, the mantle Os/Re ratio, closely constrained by the Os isotope measurements, allows tentative inferences concerning the composition of the late influx.

The ICFM requires chondritic ratios of highly siderophile elements (HSE) *a priori* only if <1% unfractionated chondritic metal and sulphide can be retained in the mantle after core formation. However, marked fractionation between solid metal (SM) and 'liquid metal' (LM; actually a metal-sulphide eutectic) is an important feature of the ICFM². Thus, the partition coefficients between SM and LM [$D(\text{SM/LM})$] are important to Os/Re fractionation. Only if the $D(\text{SM/LM})$ s for Os and Re are similar will a chondritic ratio be preserved. For Os, $D(\text{SM/LM})$ has not been measured experimentally. Data for metal and troilite (FeS) in the Odessa IA iron meteorite³ suggest a value for Os that is >50 times that of Re, although troilite may not be an ideal analogue for LM⁴.

Reference to magmatic iron meteorites (MIM) by Jones and Drake is misleading. The fractionation in MIM is between a low-Ni solid and a high-Ni liquid, with S playing only a minor role. Separation of any Fe-FeS eutectic (analogous to SM) would have taken place before the fractionation preserved in MIM⁵.

The variation of the Os/Re ratio in chondrites has been known for almost 20 years⁶, and one purpose of ref. 1 was to re-state this with modern data. Because the LIM is rigid in postulating the preservation of chondritic proportions, it allows the implications of the mantle Os/Re ratio to be explored in terms of the fine structure of chondritic composition.

To summarize, the LIM firmly predicts chondritic HSE ratios, and is supported by the isotopically derived mantle Os/Re ratio, so that its further implications may reasonably be explored. The ICFM may be able to accommodate the observed Os/Re ratio, but because many important inputs are currently unknown, its predictive value is weak.

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Age interpolation

THE equivalence of depth to age in a stratigraphic sequence can normally be established (radiometrically or palaeontologically) at only a finite number of discrete points. Age estimation between such points is impossible because sedimentary accumulation is the result of sedimentation minus erosion. The sedimentation rate (measured over short periods of time) generally far exceeds the accumulation rate (measured over long periods¹), producing a record composed of packets of rather rapidly deposited sediment separated by hiatuses (unconformities) of variable duration. Without physically based assumptions, any interpolation is generally inaccurate; moreover, an estimate of standard error at one level of a sequence will not generally apply to another.

Attempts have been made to produce depth to age conversions using a variety of assumptions (such as linear changes in deposition rate or constant rate), with the conversion produced depending critically on the assumptions²⁻⁵.

The absence of suitable radiometric markers in ancient sediments guarantees that this problem will always limit the interpretability of the geological record, yet attempts persist to surmount this geological uncertainty by using not physically based reasoning, but statistics involving 're-sampling' at a scale finer than that originally present.

Badgley *et al.*⁶ have proposed another variation on the theme, arguing that it is permissible to re-sample to a finer time scale than that of the original dated horizons. Indeed they "use the pattern of sediment accumulation for a younger portion of the sediment regime to estimate the variance in an older part of the regime; the implied assumption of stationarity is justified by the linear pattern of cumulative stratigraphic thickness over the entire Kaulial sequence". Perhaps it is, but that is essentially the point that one wishes to prove and not assume, or else a circular argument is produced.

Such 're-sampling' techniques are capricious because (1) the functional type of the re-sampling filter is chosen arbitrarily; (2) the 're-sampled' data contain noise not only from the original data but also from variations in the subjectively selected parameters of the re-sampling filter of given functional type; and (3) linear interpolations are not more, or less, accurate than any other interpolation, irrespective of the time gap between dated horizons^{2,7}.

And yet we find the claim⁶: "The standard error calculated by this resampling method can be considered a minimum value attributable to variability in sediment accumulation rate". Perhaps, but until knowledge of the true depth to time conversion is available, it is simply not possible to say whether the statistical error

is a minimum or not, all protestations to the contrary notwithstanding. Also, it is not possible to say whether the estimated stratigraphic horizon ages are systematically in error due to the underlying assumptions made.

We are surprised, and dismayed, to see the idea promulgated that one can estimate age and variance from yet another purely arbitrary interpolation scheme as proposed⁶. Playing in the cracks between the piano keys produces a tune only for the deaf.

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BADGLEY *ET AL.* REPLY—Stratigraphers often find it useful to estimate the age of a datum that lies between levels of known age. In the absence of other information, no age estimate is better than the linear interpolant. Moreover, regardless of one's theory of how sediment accumulates, the best guide to the uncertainty of any such estimate is the actual error observed for comparable quantities in comparable conditions. The primary objections of Ehrlich and Lerche are that (1) age estimation between dated levels in a stratigraphic sequence is impossible and (2) an estimate of variance in sediment accumulation rate in one stratigraphic interval should not be applied to a neighbouring interval.

If a fossil found midway between two horizons of known age were assigned an age halfway between, it would be pointless to declare that the error of this estimate is half the interval in question. But Ehrlich and Lerche imply this upper limit as the estimate of such errors ("age estimation... is impossible"). Our method reduces this unnecessarily wide uncertainty by considering errors observed for the same estimate from neighboring strata in which magnetic reversals are more closely spaced. For the "Hipparion" datum in Pakistan, we assumed that the magnitude of this error over intervals of ~1.5 Myr in the range 6.34–8.56 Myr

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would be a reasonable guide to the variability from 8.56 to 10.00 Myr. The linear relationship between sediment thickness and absolute time from 6.34 to 10.00 Myr supported this assumption, and nothing in the data contradicted it. We stand by our estimate of $\pm 90,000$ yr.

Ehrlich and Lerche are mistaken about three other points. (1) Our re-sampling involves no palaeomagnetic intervals of shorter duration than those originally measured. (2) No re-sampling 'filter' is involved. (3) Our statement that error due to all forms of uncertainty should not be systematic simply means that interpolated age estimates should be too high about as frequently as they are too low.

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Are enigmatic markings in Adelaidean of Flinders Ranges fossil ice-tracks?

SEVERAL questions may be raised concerning the provenance of markings recently described by Dyson¹ as "unequivocal metazoan remains" from the "base" of the "Wilpena Group" in the southern Flinders Ranges, South Australia. Dyson² indicated that the markings occurred in sandstones ~25 m stratigraphically below a dolomite bed representing the Nuccaleena Formation. This dolomite is the regional marker for the base of the Wilpena Group in the western Flinders Ranges³; it is discontinuous in the vicinity of the markings (near 'Wilmington') but locally reaches a thickness of ~7 m. Directly below it is 11–20 m of maroon-coloured, silty diamictite containing sub-angular to sub-rounded clasts up to 1 cm in diameter. Interlayered sandstones and siltstones at the indicated level of the markings are underlain by pink sandstones which also contain trains of exotic granules and pass laterally into sandy diamictites containing pebbles up to 3 cm. The sandstones variously show flat internal lamination and cross-bedding; stacked sets of ripple bedding outlined by heavy minerals are common and are related to linguoid current ripples and wave oscillation ripples.

These diamictites and sandstones are diagnostic facies of the Elatina Formation⁴, the glaciogenic interval of the Marinoan and upper division of the Umberatana Group.

Refrigeration during deposition of the Elatina Formation is attested by widespread varvites and common ice-rafted clasts. Pebbly arenites within the unit have been assigned a glaciofluvial or deltaic origin⁵. In a coeval terrestrial setting, laminated sandstone wedges formed in harsh periglacial conditions⁶.

Dyson¹ suggested a similarity between the markings and known Precambrian frond-like fossils such as *Pteridinium* and *Charniodiscus*, but *Pteridinium* was

dynamic conditions generated if such objects move just above the substrate⁷. Transverse shear wrinkles have been formed experimentally by passing dense suspension currents over plastic substrates⁶. 'Jigger' marks associated with parallel grooves are evidently made by floating objects touching bottom, and examples are commonly attributed to floating ice⁷. Oscillatory motions imparted to the ice by waves may lead to sets of transverse marks showing cyclical changes in width⁷. The Marinoan markings show just such changes in the width of the 'ribs' (Fig. 1) and their proximity to ice-rafted materials lends circumstantial evidence to their formation by a similar process. Comparable

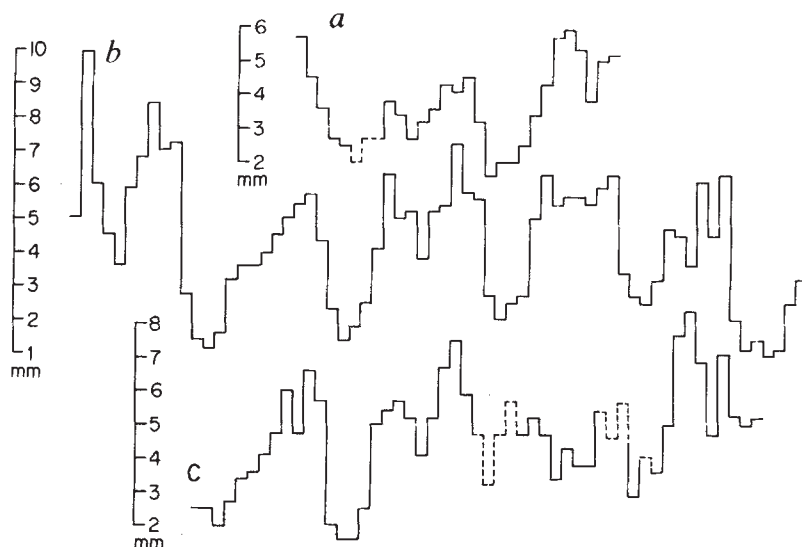


Fig. 1 Cyclical changes in width of ribs in structures described by Dyson¹ from the Flinders Ranges: a, series of ribs from left to right in marking 'a' in Dyson's Fig. 3; b, upper row of ribs in 'b'; c, lower row in 'b' shifted laterally (right) to show maximum correspondence with row above. The mean cyclicity indicated by groups of fine ribs is ~12 ribs and grooves.

evidently rather resilient or 'rubbery' and despite common folding and distortion, the transverse 'ribs' are generally quite regular in width, respectively 2–3 mm and 3–5 mm wide in several described forms. The transverse elements in *Charniodiscus* vary in width (~2–28 mm) with the size of the organism, but show a progressive gradation in dimensions along the frond. Parts of the markings show rapid changes in the width of the ribs, from 2 to 6 mm and from 1 to 10 mm wide in limited areas. Moreover, different numbers of ribs occur on either side of given intervals of the 'axis' of the 'bilateral' marking.

In size and in showing numerous nearly parallel longitudinal striae, the markings may be compared to a group of broadly intergrading inorganic sedimentary structures variously described as grooves and transverse shear wrinkles⁶, or interrupted chevron marks and striae with 'jigger' marks⁷. Linear grooves result from current-transported objects touching bottom, and chevrons may develop due to hydro-

markings formed by ice on the modern-day tidal flats of the St Lawrence, Quebec are illustrated by Dionne⁹ (his Fig. 19).

I thank Peter Haines for help with literature.

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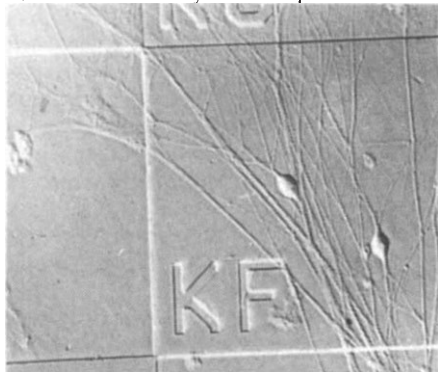
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Help in handling cells, spills and refills

Free trials for Finnpiettes, a gauge for cell aggregation and kits for chemical clean-ups round out this week's page. There are novel approaches to all kinds of laboratory needs.

A **Petri dish press** from World Products Instruments stamps plates with a grid that makes microscopic navigation easier (Reader Service No. 100). The BB Press will mark ordinary 35 mm plastic dishes



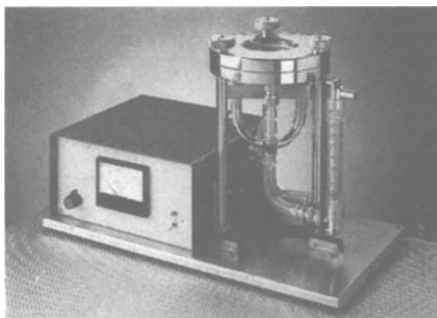
WPI's plate marker makes a good impression.

with a grid composed of 26×26 squares measuring $300 \mu\text{m}$ on a side. Each square carries a row-and-column identifier to facilitate locating areas under the microscope. The entire grid measures 8 mm on one side, and WPI says the heat-stamping procedure takes less than 2 min. The company's \$495 (US) press uses a die etched on a silicon wafer and mounted in a steel tool assembly to make its impressions.

NMR doesn't have to be an in-house job anymore. Spectral Data Services in Champaign, IL has a **commercial high-field NMR service** that includes data acquisition, interpretation and experimental design (Reader Service No. 101). SDS charges \$35 to \$50 (US) per hour to perform a variety of analyses on its 360 and 270 MHz Oxford-Nicolet systems. The company also provides consultation and simulations.

Both the physical forces and the biological interactions that influence **cell-surface adhesion** can be measured with LH Fermentation's CAMM (Reader Service No. 102). Developed by H W Fowler at the University of Bath, the Cell Adhesion Measurement Module houses a radial flow chamber that gauges adhesion by attachment in the presence of shear forces, and biological interactions by forcing cell detachment. The chamber can be autoclaved. A control unit completes the CAMM, which LH sells for about £3,000 (UK).

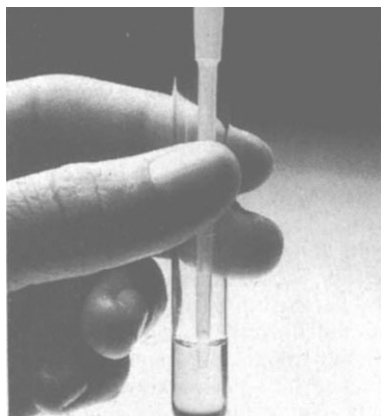
These notes are compiled by Karen Wright from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal and apply only within the country indicated.



The CAMM: perfect for sticky situations.

Safer spills are the object of a **chemical spill centre** from Helapet in Hertfordshire (Reader Service No. 103). Available at the end of October, the £295 (UK) centre is equipped with protective gear for both operators and bench surfaces and supplies chemical hazard disposal bags.

One month with a **digital Finnpiette** for free: that is the promise from Labsystems, who offers the pipettes in volumes ranging from $0.5 \mu\text{l}$ to 5 ml (Reader Servi-



A Finnpiette tip with a long reach.

ce No. 104). The company will also throw in a "generous supply" of disposable tips. Currently Labsystems is also promoting its Finntip 60 Extended tip pipette which fits most pipettes with volumes of $5 \mu\text{l}$, to $200 \mu\text{l}$, and can serve as a syringe reservoir for some stopper pipettes.

Clandon Scientific has just begun selling a **platelet aggregation recorder** that can monitor up to four channels simultaneously (Reader Service No. 105). The Aggreccorder II, a product of Kyoto Daiichi Kagaku in Japan, has a 7-inch CRT monitor and a four-colour graphic plotter for visualizing results. Priced under £9,000 (UK). Clandon's instrument uses straightforward calibration procedures and error comments.

Dynatech has just come out with

another alternative in **microtitre plate design** (Reader Service No. 106). The new Duo-Strip system consists of 16-well units moulded as two rows of 8 wells each. The strips are available in irradiated versions and U and flat-bottom formats. □

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Reader Service No.5

So few women in engineering

from Richard Pearson

As women increasingly become a major force in the labour market, their absence in certain occupations becomes even more of an anachronism.

WOMEN are noticeably absent from many engineering and technological occupations in most countries of the world. The highest representations appear to be in the Soviet Union, where women account for 40 per cent of the engineers, and Republic where they account for 30 per cent. In the United States and the United Kingdom women account for about one in ten of all engineers, rather fewer than in France where the proportion is one in six.

Is the situation changing? The proportion of women graduates has risen in the United States from 3 per cent in 1975 to well over 10 per cent in the 1980s, and from 4 to 16 per cent in the decade to 1983 in France. In the United Kingdom the proportion of women among engineering graduates has risen from 4 to over 11 per cent during the decade to 1985.

Although these proportions are increasing as a result of different mixes of changing social conditions and policy initiatives in each country, the barriers to the entry of women to the profession seem to be similar across countries. Engineering is not of course a homogeneous profession and the resultant social and attitudinal factors seem to be directing those women that are involved towards chemical and electrical and electronic engineering rather than civil or mechanical engineering — although in the United Kingdom women are poorly represented in electrical/electronic engineering. There are also major differences in enrolments by women in different institutions. One theory to support the latter variability is that a threshold of "minority" enrolments needs to be achieved before steadily increasing enrolments can be established. Thus where enrolments remain at or below 5 per cent, women in non-traditional disciplines are often labelled as "untypical", "unfeminine" and by male peers "different", putting them under pressure and inhibiting growth in enrolments. Women then have to be very single minded to succeed in such a culture. However, where enrolments can be increased to around 15 per cent, for example through the use of targeted or special measures, although women are still seen as a distinct minority, they are more accepted and can generate their own peer group support mechanisms. They then present role modelling examples leading to further growth which, once it reaches a figure of about one third representation, both staff and students regard as being a ba-

lanced representation, and minority perceptions and attitudes recede. If this is the case, then it argues for targeted policy initiatives, aimed at concentrating women's representation within a more limited number of institutions so that "critical masses" and "thresholds" can be quickly achieved and then built upon.

Earlier this year the *Employment* column looked at the role of career breaks in aiding women's employment and careers and commented on their rarity, and the broader social difficulties women face in returning to work (*Nature* 319, 522; 1986). It showed that there is still considerable resistance to women operating flexible career patterns, and that labour market factors, primarily shortages of skilled staff, are likely to prove a more significant engine of change. However, the latter presumes that women have the basic skills and education to participate in these growth and shortage occupations. Thus while career breaks can only be one relatively minor initiative aimed at helping women participate equally in working life, broader social, educational and employment policies are also needed, some of which have now been operating for a decade or more. In the United States, for example, the 1974 Women Educational Equity Act was enacted as a major initiative aimed at confronting sexism in education. Through the funding of school, college and university programmes, materials and model programmes were designed to help girls achieve educational equity. These funds were split between teachers and administrators, community groups outside the formal education system and the students themselves. Other initiatives have been started, particularly focusing on science and engineering, and a National Science Foundation directory lists over 300 projects designed to increase female participation in non-traditional fields, with the private sector providing almost a third of the funding. The focus of most projects was on engineering, mathematics or general science subjects, and over 80 per cent were based in universities and colleges.

Residential summer schools are also now a common form of initiative with girls attending 2-4 week workshops giving overviews of engineering, specialist teaching in mathematics and computing, industrial field visits and practical experience through project work. Industrial companies are major participants in these

programmes providing both general funding and direct assistance in the form of projects, advice and teaching staff. Direct work with schools and students is also seen as important both in generating awareness of careers in engineering and in providing more direct counselling. Within higher education itself, various support schemes operate to help and encourage women to pursue engineering courses, while other schemes seek to encourage them to enrol on engineering courses after more general first-year programmes. More radically, dual degree programmes now exist which enable women in a liberal arts college to transfer to an engineering degree in another college in their third year, by which time they have developed the intellectual and social skills to enable them to participate equally on "male dominated" engineering courses. Finally the establishment of women's professional support groups and the use of female role models are all further helping the process of enhancing women's participation in science and engineering. In the United Kingdom the biggest initiative to date has been Women in Science and Engineering Year in 1984 (*Nature* 315, 84; 1984) which gave further impetus to a broad range of initiatives already under way which are aimed at raising the profile of engineering and science careers for girls, although there were no significant injections of cash to aid the process. A limited number of scholarships have, however, been paid to help girls acquire the necessary training and further education to become technicians. After a slow start and limited impact, the equal opportunity legislation of the early 1970s is now at last seen to be having an impact.

In the West at least, women are still significantly under-represented in engineering and technology. Progress is being made under a mixture of market forces and individual initiatives, but it is likely to be some decades before equality is in prospect. The process can only be accelerated by action across a wide range of fronts, including perhaps a national policy commitment, funding for affirmative action programmes, and special counselling and experience schemes aimed at school, higher education and the labour market, as well as in the home and society at large. □

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